Johnston

PHYSIOLOGICAL REVIEWS

EDITED FOR

THE AMERICAN PHYSIOLOGICAL SOCIETY

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VOL. VI, NO. 4

Issued October, 1926

BALTIMORE, U. S. A.

Entered as second-class matter, January 15, 1921, at the Post Office at Baltimore, Md., under the Act of March 8, 1879

Made in United States of America

PHYSIOLOGICAL REVIEWS

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Subscriptions will be received in advance only. The subscription price is:

$6.00 per volume in the United States, net postpaid
$6.25 per volume in Canada, net postpaid
$6.50 per volume elsewhere, net postpaid
$2.50 per single number, net postpaid

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PHYSIOLOGICAL REVIEWS

VOL. VI OCTOBER, 1926 No. 4

THE CONDUCTION OF THE NERVE IMPULSE

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When a sense organ or a nerve trunk is stimulated, a muscle or gland located at a considerable distance may become active, and this activity may readily be shown to be dependent upon the nervous connections. We say that a nerve impulse has traversed the nerve and initiated the activity of the effector. The exact nature of the impulse is still a matter of speculation, and although a considerable body of evidence in favor of an electrical theory has now accumulated, as recently reviewed by Lillie (1922, 1923), it is premature to identify the impulse with the electrical disturbance. The emphasis on the electrical aspect is due partly to the relatively enormous sensitivity of our electrical recording devices which makes study of these phenomena comparatively easy; while the accompanying increase of metabolism and heat production have only recently been measured. Our definition of the nerve impulse therefore must be in terms of the fundamental physiological observation, and may be briefly stated as a *conducted tendency to excite* (cf. Davis and Brunswick, 1926).

It is our present purpose to review recent experimental work bearing on the phenomenon of conduction, and, in view of the large volume of material, to select for special emphasis those investigations which appear to give a better understanding of the intimate mechanism.

Our first concern, as Lucas pointed out in 1917, is to determine the type of disturbance to which the nerve impulse belongs. Although the energy liberation of the impulse is so slight as to make investigation extremely difficult, we may be sure that the impulse delivers energy to the effector, for the latter is stimulated to activity by the impulse, and stimulation in general requires the expenditure of energy. What form of energy is it? And is the energy transmitted passively along the nerve

like a sound wave down a speaking tube, or does the nerve actively participate like a burning train of gunpowder? We may hope to answer the latter question if we can find a method for measuring the impulse at various points along its course, for in the latter case the impulse might under suitable circumstances increase as it is transmitted, but in the former case any increase would be impossible. This is exactly the argument followed by Lucas (1917), who believed that the impulse could be measured satisfactorily by setting it to face a region of impaired conduction or "decrement". From the well-known experiments by this method Adrian (1912) concluded that the impulse recuperated after emergence from a region of depression, and he definitely placed nerve conduction in the second class of "active" or "contributory" conduction. This implied an all-or-none behavior of the nerve, i.e., that each point along the nerve contributed to the maintenance of the disturbance the maximum energy of which it was capable; and this principle, also supported by other types of experiment, has become the corner-stone of nerve physiology. Recently, however, the adequacy of the method employed by Adrian, and the whole conception of decrement underlying it, has been called into question. We shall therefore begin by a consideration of "decrement" and of evidence for the all-or-none principle apart from Adrian's experiment. Then we shall proceed to metabolism and heat production and finally to the electric response, excitation, recovery, and certain speculations as to the nature of the nerve impulse.

THE QUESTION OF DECREMENT AND THE ALL-OR-NONE PRINCIPLE. The work of Kato and his collaborators (1924a, 1926) is of special importance, not only because they were the first to reopen the question of decrement, but also because of the wealth of experimental evidence which they presented. They repeated the experiments on which the theory of conduction with a decrement was based, and, while verifying the original observations, found serious flaws in both technique and interpretation. Early experiments by Werigo (1899), Dendrinos (1902), and Fröhlich (1904a) had shown that the duration of action of a narcotic necessary to abolish conduction depended on the length of the narcotized region. From this it was concluded that the impulse became progressively weaker as it traversed the narcotized portion of nerve. This is the classical conception of "decrement" (cf. Verworn, 1914; Lucas, 1917). The experiments were as a rule performed on the sciatic nerve of the frog, which offers only one or two centimeters length for narcotization. Frequently chambers as small as three or four milli-

meters in length were employed, and the pronounced delays in the action of narcotics were found with these very short lengths. Kato, studying the large Japanese toad, was able to use chambers three and four centimeters long. He confirmed the greater resistance to narcotization of lengths less than six millimeters, but beyond this found no correlation between length of nerve and time of failure of conduction. A great variety of narcotics, such as alcohol, chloroform, ether, chloral hydrate, urethane, cocaine, morphine, ammonia, etc., as well as heat and cold, were employed, and always with the same result. The best nerves for such experiments are those which give off no branches but remain of uniform cross-section such as *n. abdominalis* or the bundle, dissected out of the sciatic trunk, serving the *flexor digitorum* group of muscles. When two such nerves from the same animal were mounted, one lengthwise and the other crosswise, in an oblong chamber, Kato found that the differences in time of failure as between a long and a short region fell clearly within the range of experimental error. In fact the differences, when plotted statistically, form a perfect distribution curve about zero as a mean. To explain the divergence of the short lengths, Kato in his first monograph postulated a diffusion of narcotic out of the chamber along the nerve and a diffusion inwards of normal tissue fluids, giving a gradient of concentration of narcotic so that full depth of narcosis is only reached at a distance of 3 mm. from the wall. Davis, Forbes, Brunswick and Hopkins (1926) criticized this interpretation, particularly as to the inward diffusion of normal fluids, and proposed an alternative view akin to that originally suggested by Werigo (1899). Their interpretation will be considered below, but Kato in his second monograph (1926) has presented direct evidence for his postulated gradient of narcosis by showing that the threshold to a sharply localized mechanicl stimulus follows just such a gradient. His explanation is therefore presumably correct; but it should be noted that his gradient is demonstrated on a whole nerve trunk and that it does not necessarily exist in *all of the individual fibers*, but may be only in those centrally located in the trunk. Such fibers would be more shielded from access of narcotic near the wall than they are in the middle of the chamber, and we do not need to assume inward diffusion of normal fluids to any marked degree.

Another line of evidence favoring decrement, which Kato has shown to be fallacious, is that followed by Lodholz (1913), Rehorn (1914), and Adrian and Forbes (1922). In brief, they found that at the moment when conduction from stimuli applied above the narcotizing chamber

just failed to elicit a response, electrical stimuli applied at various points within the chamber would still be successful. The threshold within the narcotized area was abnormally high, to be sure, but it had risen considerably before conduction failed; and importance was attached to the fact that after failure of conduction the threshold was higher the greater the length of narcotized nerve that lay between electrode and muscle. The usual interpretation was that the stronger stimulus set up a larger response which was able to traverse the greater length of narcotized nerve. This implies a different type of behavior in narcosis from the usual all-or-none of normal nerve, and Verworn (1914) employed the terms "isobol" and "heterobol" to designate the normal and abnormal states. To rule out the possibility that the result was due to spread of stimulating current to normal nerve outside the chamber the nerve was crushed between the electrodes and the distal chamber wall. Thereafter stimuli much stronger than those previously effective caused no response. It was assumed that this proved that the stimuli had not been "spreading" and taking effect outside of the chamber, but Kato shows that this assumption is unjustified. It constitutes a basic fallacy in Lucas' argument (1917).

Various types of evidence are advanced by Kato on this point. He determines the point at which the impulse arises by first measuring the velocity of conduction in the nerve by the familiar Helmholtz method and then observing the shortening of the latent period that results from increasing the strength of stimulus. The differences in latency are far beyond experimental error, and indicate that stimuli which are apparently not excessively strong may take effect as far as 30 mm. from the electrodes. A comparison with the latency of mechanical stimulation (cutting the nerve) shows that spread is negligible with just maximal stimuli. Unfortunately Kato usually expresses his stimuli merely as "coil distance", so that exact evaluation of the spread is impossible. In his second monograph he describes an experiment by Adrian's (1914b) method of observing the change in the least interval for muscular summation with the second stimulus applied close to the muscle, and the first, whose strength is varied, applied at a distance. In this case stimuli are measured as the reciprocal of the resistance in the primary circuit, and it appears that a stimulus ten times the maximal took effect 19 mm. from the electrode. Such spread may also occur with fluid electrodes such as Lucas employed. Cooper (1926) points out that these excessive spreads are measured on bull-frog nerves with a heavy outer sheath which Bishop, Erlanger and Gasser (1926e; cf. also Erlanger

and Gasser, 1924) show is an important factor in current spread. Kato does distinguish between "inside" and "outside" spread. The former sets up an impulse in the fiber to which the stimulus is applied, and is not influenced by various short-circuiting or grounding procedures, while the latter is the type which may set up an impulse in other fibers than those to which the stimulus is applied. Crushing the nerve interferes with both types.

Kato repeated experiments of the Lodholz and Rehorn type using mechanical stimuli instead of electrical, and it appears that when conduction fails for impulses initiated above the chamber they also fail for stimuli of all strengths at all points within the chamber. Even cutting, crushing, or burning causes no response.

Lucas (1913) studied the effect of alcohol on the nerve with particular reference to the prolongation of the least interval after a previous stimulus at which a second stimulus can set up an impulse capable of reaching the muscle. This interval is markedly increased when the nerve is narcotized. Lucas' data indicate that it is more prolonged the greater the length of narcotized nerve the impulse has to traverse, and he concluded that the result was due to decremental conduction which extinguished small impulses set up early in the relative refractory phase while allowing larger ones, set up somewhat later, to pass. The correlation between length of least interval and length of nerve makes this seem probable, but both Davis, Forbes, Brunswick and Hopkins (1926) and Kato (1926) point out that the result may well be due to spread of stimulus. Kato (1924a), controlling this factor, finds no correlation. The prolongation of least interval is marked, whether the stimuli are applied in the chamber or above, and Kato explains this as due to a slowing of the recovery process. The fact that the least interval may be prolonged much beyond the time necessary for complete recovery in normal nerve shows that this must be the case.

Cooper (1926) has also repeated the experiments with control of current spread and confirms Lucas' original observations. Not only is there a correlation between the increase in least interval and length of nerve, but conduction always fails first from the more distant electrode. This is contrary to Kato's numerous observations, and suggests that the cause of the discrepancy may possibly lie in a greater permeability of the outer sheath of Cooper's green frog nerves in the upper thigh and lumbar plexus as compared with the lower portions, or in differences in diameter of the trunk.

Ishikawa (1924) reports that the rate of extinction of conduction may

vary with the section of nerve trunk selected, although the length of nerve exposed is the same in each case; and preliminary experiments in this laboratory confirm this observation.

Ishikawa (1924) offers certain criticisms of Kato's work, but it is difficult to follow the logic of his argument or to evaluate his observations from the meager descriptions available.

Lucas (1913) concluded that the rate of recovery was unaltered by alcohol, since the least interval for muscular summation for a stimulus just double the threshold value remains constant in narcosis. Davis, Forbes, Brunswick and Hopkins (1926) point out the logical fallacy of measuring the stimulus by which recovery is to be tested in terms of the threshold stimulus, which rises in narcosis. Kato (1926) has repeated Lucas' experiment and verifies his results, but finds that, when any multiple of the threshold other than two is chosen as test stimulus, by Lucas' own criterion the recovery process is slowed in narcosis. In the case of double the threshold there is apparently a chance balancing of two opposing factors: 1, the slowing of recovery, and 2, the increase in strength of the test stimulus corresponding to the rise in threshold. Lucas' result is therefore not valid evidence for decremental conduction.

A third method used by Lucas (1913) was to measure the least interval between two stimuli which would just give muscular summation, and then interpolate a third stimulus between them. The least interval was lengthened, and Lucas inferred that the interpolated stimulus set up a subnormal impulse which died out because of decremental conduction but nevertheless left a brief refractory phase behind it. Kato (1926) gives strong evidence that Lucas' results are due to a local depressing effect of the rather strong interpolated stimulus. He makes the important observation that the refractory period has the same duration after a subnormal impulse travelling in the relative refractory phase of a predecessor as it has following a full-sized impulse. This in itself is strong evidence for an all-or-none behavior, since it suggests a reaction which runs to a definite limit whatever the state of the system when the reaction is initiated. When this limit has been reached recovery from it follows the same course whether the energy liberation was large or small.

Decrement in rate of conduction of the impulse in narcosis has been described by Fröhlich (1904b), but his data are very meager, and Koike (1911), in a more extensive investigation of the question, comes to the opposite conclusion. Koike is criticized by Fröhlich (1912) as having too great an experimental error to justify his conclusions. Adrian (1914b) made more accurate measurements of conduction velocity,

timing the arrival of the impulse below the narcotized region by measuring the interval required for the nerve to recover sufficiently to conduct a second impulse which would evoke a summated contraction. The end-point is quite sharp and the method seems better than measurements of the latency of contraction or even of muscular action currents. Adrian's results show quite clearly that there is no progressive slowing of the impulse in a narcotized region. Kato (1924a) has confirmed this finding, using the method of latency of muscular contraction. The probable error of his results is less than Koike's or Fröhlich's because of the greater length of nerve subjected to narcosis. Achelis (1922) on the other hand, reports a decrement in conduction velocity, but his probable error is comparatively large, and Davis, Forbes, Brunswick and Hopkins (1926) have indicated two possible sources of error in his method; namely, spread of stimulus, and a more rapid diffusion of narcotic into the nerve in the vicinity of the cut end. Seliškar (1926) without giving details, reports no decrement in the case of cardiac muscle from the turtle's auricle treated with alcohol. Drury (1924, 1925) finds definite evidence of progressive slowing in auricular tissue subjected to compression, but the distances employed were only a few millimeters, and it is possible that the compression did not affect the muscle uniformly, or that it merely disorganized the syncytial arrangement of the fibers. At present the balance of evidence is clearly in favor of a constant although reduced rate of conduction in narcotized nerve.

Decrement in the electric response has been reported by earlier workers (Boruttau and Fröhlich, 1904); but in these experiments no control was employed to show that some of the fibers did not cease conducting entirely, some near the upper end of the chamber and some near the middle, and the progressive diminution may easily be explained in this way. Beritoff (1923) called attention to this cause of apparent decrement in the electric response, and also to diffusion of narcotic along the nerve, but apparently he believed that true decrement occurred as well. Apparent decrement may also be seen as the nerve approaches a region of injury. Davis and Brunswick (1926) describe such depression of response extending 15 to 20 mm. from a crush. The corresponding alterations of threshold near an injury are a matter of text-book knowledge, and Koch (1924, 1925) describes a parallel gradient of the demarcation potential. Alterations of electric response in regions of polarization, first formulated by Hermann and studied in most detail by Bishop and Erlanger (1926f), are fundamentally similar. All depend on gra-

dients preëxisting in the tissue, like Kato's gradient of narcosis; and, unlike Lucas' "conduction with a decrement", they are quite compatible with the all-or-none law.

To avoid confusion from fibers ceasing to function, Kato (1924a) and Davis, Forbes, Brunswick and Hopkins (1926) recorded as a control the action current from normal nerve beyond the narcotized region and found that while the response here remained full-sized, showing that all fibers were still conducting, the response within the narcotizing chamber could be considerably diminished, *but to an equal extent at all points*. Kato tacitly accepts the electric disturbance as a measure of the size of the impulse, and Davis, Forbes, Brunswick and Hopkins also accept it after a theoretical discussion. But in any case there seems little doubt that the electric disturbance itself suffers no progressive decrement, and further, that it may increase again to normal size as the impulse passes from narcotized to normal nerve. This indicates definitely that the electric disturbance is all-or-none in character and that its source of energy is in the fiber itself. This is supported by Adrian and Zotterman's (1926) beautiful experiment showing an all-or-none relationship between the (stretching) stimulus applied to a sensory end-organ in muscle and the magnitude of the resulting electric disturbances in the individual sensory nerve fiber.

While the all-or-none law is thus proved for the electric response, the logical difficulty of accepting this as proof of the all-or-none character of the impulse still remains. Either we must show that the electric disturbance is an essential element in the mechanism of conduction or we must find some other method of obtaining a measure of the impulse. Davis, Forbes, Brunswick and Hopkins (1926) discuss this question at some length, and conclude, partly by a process of elimination and partly on the basis of the evidence in favor of an electrical mechanism of conduction (cf. Lillie, 1923; Cremer, 1909; and discussion to follow) that the experiments described constitute the best present evidence for the all-or-none character of the impulse. Powerful supporting evidence for the all-or-none principle is to be found in Adrian's (1914a) observation that, as narcosis proceeds, the maximal and minimal stimuli at a point above the narcosis gradually converge, suggesting that in the individual fiber the size of the impulse is independent of the strength of stimulus. Furthermore, as Adrian points out (loc. cit.), all-or-none behavior is almost a necessary consequence of the existence of a threshold of stimulation and a refractory phase. But these considerations do not in themselves constitute proof of all-or-none behavior under all conditions.

Adrian himself believed in conduction with a decrement in narcosis, which implied a departure from all-or-none behavior. It is important to note that the experiments on electric response show an all-or-none behavior in narcosis as well as in normal nerve.

Kato concludes that there is no such thing as decremental conduction and that the all-or-none law is universally valid, but Davis, Forbes, Brunswick and Hopkins point out that on theoretical grounds, we must expect a "transitional decrement" for a short distance whenever the impulse passes from a region of normal to a region of depressed response, quite irrespective of any gradient of narcosis such as Kato considers. Their argument may be outlined in brief as follows. The nerve impulse must be measured in terms of energy, since energy is required to stimulate. The problem of conduction is a problem of the nature of this energy, its liberation, its spread, and its excitatory effects. There may be liberation of energy by successive portions of nerve sufficient to compensate for the inevitable losses; which would give all-or-none behavior. If the energy liberation by the nerve were nil we should be dealing with the passive type of conduction, which is inevitably decremental. If there is energy liberation we may analyze the conduction into three phases: 1, the liberation of energy by the nerve, which we shall term the "fundamental reaction" or "local response", 2, the spread of this energy to neighboring regions; and 3, the initiation of the local response in these neighboring regions. This last obviously corresponds to Lucas's "local excitatory process". We usually find in addition a fourth phase, a recovery process. Part of the energy available at a given point represents the energy liberated there by the local response, and part spreads to it from neighboring active points. The "nerve impulse" is the sum of these parts; i.e., the total energy available for excitation. Now if, given three successive points, the local response is greater at the first than at the second and third, then the total energy available at the second point will be greater than that at the third, since the quantity of energy spreading from the first to the second will be greater than that from the second to the third. The impulse will therefore become progressively weaker until it has gone so far as to be beyond the limit of spread from the first point. Therefore there must be "transitional decrement" even although the *local response* at every individual point on the nerve is strictly all-or-none. How great the limit of spread may be the authors do not state, except to indicate that experiments show that it cannot be more than five or six millimeters. They suggest that it may explain the results of Drury (1924, 1925) who reports decrement in compressed auricular tissue.

Broemser (1925), in elaborating a theory of conduction as a wave of osmotic pressure change, (see p. 582, below) shows clearly how such transitional decrement would be a necessary consequence of his type of mechanism. He also considers another conceivable type of decrement, and also increment, which would occur if the local response were not all-or-none but were proportional to some function of the amount of energy reaching a given point from its neighbor. If the liberation were less than enough to balance the losses, a true decremental conduction would result, corresponding to the damping of a wave. If the liberation were in excess of losses, conduction with an increment would occur. It may be said at once, however, that there is no experimental evidence in the case of nerve for either of these two last possibilities, although the decremental conduction during the recovery period in Lillie's iron wire model (1925) may be an illustration of the first case. The subnormal impulses found in muscle by Dittler and Oinuma (1911) by electrical methods are almost certainly due to the diminution in electric response on slight shortening of a slack muscle as described by Fulton (1925). Apparently the local response in nerve and skeletal muscle adheres strictly to the all-or-none law.

Since there is no valid evidence for decremental conduction, except perhaps as a transition phenomenon, and since the electric disturbance (and presumably the impulse itself) increases in size after emerging from a region of depression, we may safely conclude that conduction is of the active "contributory" type, analogous to a burning train of gunpowder.

METABOLISM OF NERVE. Study of the local response is made difficult by the extreme efficiency and economy of the whole process. We are now, however, possessed of certain definite data in regard to both heat production and gaseous metabolism which are of fundamental importance.

Heat production. Hill in 1911 was unable to detect the heat associated with the nerve impulse, and was forced to the conclusion that it was less than that required to raise the temperature of the nerve by 1×10^{-8}°C. Recently Downing, Gerard, and Hill (1926) have reinvestigated the question with improved methods, and have found an initial heat production slightly above the figure just quoted. A frog's sciatic nerve stimulated 280 times a second shows an immediate heat production of 7.6×10^{-6} cal. per gram per second. This corresponds to a rise in temperature of about 2.7×10^{-8}°C. per impulse. This represents only about one-tenth of the total heat, however, for heat production continues for ten minutes after stimulation (at 15°C.), giving a total of 6.9×10^{-5} cal.

per gram of nerve per second of stimulation. Apparently there are "initial" and "recovery" phases, corresponding to those in muscle, although future experiments must decide whether the first is anaerobic and the second aerobic, as the analogy suggests. The heat production per impulse is not constant, but falls off very definitely during a period of tetanic stimulation, reaching half of its original value in a few minutes. This point must be remembered when comparing heat data with the fuel consumption or the production of metabolites. If we assume oxidation of carbohydrate, the total heat figure cited above corresponds to a utilization of 0.0008 cc. of O_2 per gram per minute of stimulation, and an equal production of CO_2. The energy per impulse per centimeter of single fiber is about 10^{-5} erg, which is far more than enough to account for the energy of the electric disturbance. It should be noted that all of these figures refer to the *increase in activity associated with the impulse*, since the method depends on changes in temperature, and gives no indication of the resting metabolism.

Oxygen utilization. That oxygen is utilized in the conduction of the impulse has been recognized since Thörner (1908) showed that a nerve in the absence of oxygen ceases to conduct sooner when stimulated than when allowed to rest. An asphyxiated nerve will recover its function on admission of oxygen, although Fillié (1908), Gottschalk (1919), and Cooper (1923) all note that the recovery is incomplete unless the nerve is also washed in fresh Ringer's solution, which presumably removes accumulated products of metabolism. Thörner (1909) and Gottschalk (1919) both note the favorable effect of washing, even with a strictly oxygen-free solution. Fillié found that the lower limit of oxygen content in Ringer's solution which would maintain function is about 0.3 mgm. of O_2 per liter. This corresponds to a partial pressure of roughly 5 mm. of Hg. Several attempts have been made to determine the rate of consumption of oxygen. Haberlandt (1911) and Adam (1921), using microspirometers, both found a resting consumption of about 0.0014 mgm. O_2 per gram of nerve per minute. Both reported a slightly increased consumption on stimulation which it was impossible to measure on account of irregular behavior of the apparatus. Buytendyk (1910a and b) also noted a definite increase on stimulation, averaging about 50 per cent of the resting output, in the case of the *n. trigeminus* of *Gadus Morrhua*. His figures show considerable variations, particularly as to resting output, and he was unable to demonstrate any increase on stimulation of the frog's sciatic. Haberlandt reported a respiratory quotient of approximately unity for both resting and stim-

ulated nerve. Sheaff (1922), using an involved microchemical method, found a resting consumption of 0.0434 to 0.076 mgm. per gram per minute, and an increase to 0.13 to 0.15 mgm. per gram per minute during stimulation: an increase of roughly 200 per cent. In table 1 the above data are presented calculated in terms of carbon dioxide pro-

TABLE 1

Metabolism of frog's sciatic nerve, reduced to milligrams of carbon dioxide per gram of whole nerve per minute

INVESTIGATOR	YEAR	METHOD	TEMPERATURE	CO_2 OUTPUT; MILLIGRAMS PER GRAM PER MINUTE*	
				Resting output	Increase on stimulation
			°C.		
Thunberg	1904	Microspirometer	16°	0.00057	
Tashiro	1913	"Biometer"	19.5°	0.0055	0.0087
Riggs	1919	"Biometer"	20°	0.0033	0.004†
Moore	1919	Indicator	18.5°	+	−
Parker	1925	Indicator	22°	0.0055	0.00077
Haberlandt	1911	O_2 consumption	21.5°‡	0.002	+
Adam	1921	O_2 consumption	22°	0.002	<0.0025
Sheaff	1922	O_2 consumption	22.5°	0.06	0.08
Hirschberg and Winterstein	1919	Sugar and protein utilization	±22°	0.0023 to 0.008	0.01
Downing, Gerard and Hill	1926	Heat production	15°		0.0016

* Many of these figures are averages of several values from the same worker, and are to be regarded as approximations only.

† Stimulation by previous immersion in Na_2SO_4.

‡ Average. Range is 19°–24°.

duction (assuming R. Q. = 1) for comparison with other metabolic determinations.

Carbon dioxide production. The production of carbon dioxide by resting nerve was measured by Thunberg (1904) by means of a microspirometer. His low value (cf. table 1) of 0.00057 mgm. CO_2 per gram per minute is to be explained in part by the relatively low temperature (16°C.) of his experiments. Tashiro (1913), using his "biometer",

reported a resting production equivalent to 0.0055 mgm. of CO_2 per gram of nerve per minute and an increase on stimulation of 100 to 200 per cent. Bayliss (1915) suggested that this large increase on stimulation might be due to heating by the stimulating current, but Riggs (1919) confirms Tashiro, stimulating chemically by immersing in sodium sulphate solution. A serious criticism of the "biometer" has been made by Adam (1921) who attempted to use it and found that the formation or non-formation of the crystals of barium carbonate on the surface of the drop of barium hydroxide is largely conditioned by the presence or absence of dust particles in the air. He concludes that the method, while good qualitatively, is unreliable quantitatively.

Moore (1918, 1919), using a colorimetric method, sensitive to 10 per cent at best, was able to demonstrate a resting production of carbon dioxide which was increased by injury but was apparently unaffected by stimulation. Parker (1925a) employed a modified Osterhout respiration apparatus (1918), calibrated by the use of known concentrations of alkali in the indicator solution. Owing to errors in calculation[1] in the paper by Ray (1924) on which the calibration of the apparatus is based, Parker's values are too large and should be multiplied by the factor 0.619 for the experiments at 22°C. and 0.623 for those at 23°C. We shall apply this correction to such figures as we cite from his papers.

Parker finds that the lateral-line nerve of the dog-fish, and also the sciatic of the frog (1925c), when first placed in the chamber, unstimulated, both show a relatively rapid initial production of carbon dioxide, falling off to a nearly steady rate after a half an hour for the dog-fish or an hour and a half for the frog. The initial gush is probably to be regarded as the escape of gas dissolved in the tissues, and also as a result of degenerative processes at the cut ends, for the gush can be renewed by cutting or crushing freshly. Parker regards the CO_2 output during the "steady state" as the product of metabolism. This rate is nearly the same for both nerves, and averages 0.0055 mgm. of CO_2 per gram of nerve per minute for the frog's sciatic at 22°C. This is identical with Tashiro's figure. Parker determined in a similar fashion the respiratory rate of pure connective tissue of the frog, and found it to be 0.0060 mgm. CO_2 per gram per minute. Since fascia forms only approximately 43 per cent of the mass of the nerve trunk

[1] The theory on which these calculations are based, as suggested by Dr. E. J. Cohn, is entirely sound, the errors being in Ray's numerical application. The author is indebted to Mr. H. Blum for calling attention to the errors.

(Parker, 1925d) it is obvious that the whole CO_2 output cannot be attributed to the fascia, but the nerve fibers must contribute 0.0050 mgm. CO_2 per gram of strictly nervous tissue. He further calculates that one centimeter of resting nerve fiber from the lateral-line nerve of the dog-fish excretes about 2.6×10^{-8} mgm. CO_2 per minute. Electrodes for stimulation are applied outside the chamber to avoid heating and electrolysis. The rate of CO_2 production is measured during alternate periods of rest and tetanic stimulation. The results are averaged in such a way as to avoid error from the continued slight decrease in resting production. The gross average increase is about 15 per cent for both frog and dog-fish. For the frog the absolute increase is about 0.00077 mgm. of CO_2 per gram of whole nerve. Since control tests show that fascia does not increase its output on stimulation it is evident that the strictly nervous tissue must increase its activity by 25 to 30 per cent on stimulation.[2]

The nerve cord of the lobster containing many ganglion cells was also investigated (Parker, 1925b), and found to produce carbon dioxide at a far higher initial rate than nerve trunk, but the rate fell so rapidly and continuously that it was impossible to determine a characteristic "level" of production.

Fenn (1926a) has described a method for measuring the CO_2 output of nerve which depends on the alteration of conductivity of a barium hydroxide solution, and successfully avoids the irregularities of crystal formation which interfere with Tashiro's biometer. He claims a sensitivity equal to Tashiro's and ten times as great as Parker's. Preliminary results (Fenn, 1926b) indicate a 10 per cent increase in CO_2 output during recovery from prolonged stimulation, although the immediate increase due to stimulation is small and irregular.

Comparing the resting CO_2 output with the oxygen consumption, and assuming a respiratory quotient of unity, we find Sheaff's figures about ten times as great as we should anticipate. Adam's figures, however, are of the proper order of magnitude, as they correspond to an average CO_2 production of 0.0020 mgm. per gram per minute.

Tashiro and Adams (1914) found a qualitative relation between oxygen and carbon dioxide, for in an atmosphere of hydrogen the resting CO_2 output was diminished and no increase was observed on stimulation.

In contrast to the agreement as to resting CO_2 production there is wide divergence between Tashiro and Parker as to the extent of increase

[2] Parker's figure of 16 per cent is obviously a mathematical error.

on stimulation. Hill's heat production corresponds to the formation by oxidation of carbohydrate of 0.0016 mgm. CO_2 per gram of nerve per minute of stimulation. This is about double Parker's figure, but far less than Tashiro's 200 per cent increase. It is to be expected that Parker's figures would be too low because any lag in diffusion of carbon dioxide would cause part of the excess to appear after, rather than during, stimulation. Furthermore, the very prolonged heat production found by Hill indicates that such a lag may be not only in the diffusion but in the actual formation of the gas. We have already noted the fall in heat production during tetanic stimulation (Gerard, 1926). Since Parker stimulated for about ten minutes at a time we should expect a comparatively low figure for his CO_2 output. A closer comparison cannot be attempted because Parker, to judge from the type of induction coil which he employed, used a frequency of stimulation not more than half as great as that of Downing, Gerard, and Hill, while his experimental temperature was eight degrees higher. Satisfactory correction cannot as yet be made for these differences, but on the whole we may regard Parker's carbon dioxide figures as quite compatible with those for heat production.

Ammonia production. The production of ammonia by nerve, increasing on stimulation, is reported by Tashiro (1922) and confirmed by Winterstein and Hirschberg (1925). The amount is extremely small, Tashiro detecting 0.00032 mgm. NH_3 per gram of frog's sciatic per minute a few minutes after excision. The output falls off in the course of time on stimulation but there is an increase of 0.00036 mgm. NH_3 per gram per minute. Winterstein and Hirschberg allow their experiments to run for several hours to obtain larger quantities for measurement, and report similar results. Large and unexplained differences are noted, however, depending on whether the nerve is suspended in air or immersed in fluid. Parker (1925a) expresses doubt as to this ammonia production, but has not yet published his evidence. In regard to the whole question we must emphasize the minute quantities measured (less than one-tenth of the carbon dioxide output) and the notorious difficulties besetting accurate ammonia determinations. In view of this, one cannot profitably discuss the possible significance of these findings, whether representing post-mortem decompositions, diffusion from a pre-formed store, or true nervous metabolism.

Fuel consumption. The metabolism of resting and stimulated nerve has been studied from the point of view of fuel consumption by Hirschberg and Winterstein (1919). The figures are only approximate, due

to the small amounts of material available, and it is not clear what precautions were taken against bacterial contamination. Neither are there any controls as to the physiological condition of the tissue after the 24-hour experiment. It is of some interest, however, to compare their figures with those for CO_2 output. Sugar (dextrose) is taken up from a 0.5 per cent solution at a rate averaging 0.00243 mgm. per gram of nerve per minute, at 20 to 24°C., which is equivalent to 0.0036 mgm. CO_2. In the absence of sugar there is a pronounced disappearance of fat, but dextrose acts as an efficient "fat sparer". Nitrogenous material may also be utilized, to the extent of 0.007 mgm. of protein per gram per minute, but in the presence of sugar this falls to 0.0023 mgm. On the extreme assumption that all of the carbon in this protein appears as carbon dioxide, the maximum total CO_2 output in presence of sugar would be about 0.008 mgm. per gram per minute, which is in good agreement with the direct determinations. Hirschberg and Winterstein find that carbohydrate, fat, and protein metabolism are all dependent on the presence of oxygen. On stimulation they find a very large increase in fuel consumption; about 500 per cent for sugar, 300 per cent for fat (in absence of sugar), and 200 to 300 per cent for protein (in absence of sugar). Results qualitatively similar are obtained with the entire spinal cord. It is apparent that the increases on stimulation are in line with Tashiro's results, but contrary to those of Parker, Moore, Fenn and Hill.

Boruttau (1920), in a brief note, states that nerves from starved animals continue to function longer and show fewer signs of fatigue if sugar to the extent of 5 per cent is added to the Ringer's solution in which they are kept. The effect of sugar is not marked if the animals are well nourished.

THE CHARACTER OF THE FUNDAMENTAL REACTION. The general inference from the work on nerve metabolism is that the conduction of the nerve impulse involves a chemical mechanism similar to those encountered in other tissues, and that probably the typical reaction is an oxidation of carbohydrate to carbon dioxide and water. The various steps in the mechanism are, however, far from clear.

The rôle of oxygen. Gottschalk (1919) considers the function of oxygen to be the removal of toxic products of metabolism and considers energy development in the nerve to be an anaerobic process. This conclusion is based on the relation between the time an asphyxiated nerve is allowed to recover in air and the time required for a second asphyxiation. For a brief recovery of less than eight minutes

the times are proportional to one another (Gottschalk, 1914) but for longer periods they are independent. This suggests that the inward diffusion of oxygen is practically complete in eight minutes, and he argues that when the nerve is being asphyxiated the outward diffusion of dissolved (or otherwise stored) oxygen will be complete in the same time. But a nerve will conduct in an atmosphere of nitrogen for an hour or more. For the greater part of the time it must be functioning anaerobically, and it is unlikely that in the course of asphyxia the character of its fundamental reaction alters radically.

Forbes and Ray (1923) show that a mammalian nerve kept at a temperature low enough to abolish conduction will survive (i.e., resume function on warming again) much longer than a nerve kept at room temperature. They attribute the longer survival to the slowing of metabolism. Similar suspension of function associated with longer survival may be produced by keeping the nerve *in situ* for a time after death. The slowing of metabolism in this case is attributed to lack of oxygen or to accumulation of carbon dioxide. Gottschalk (1921) offers a similar explanation for his observation that bathing a nerve in hypotonic sodium chloride solution increases its resistance to subsequent asphyxia. He believes that the increased water content of the nerve slows its metabolism. Cooper has studied the effects of excess of carbon dioxide (1924) and of asphyxia (1923). The carbon dioxide evidently slows recovery and, in common with other acids, produces a marked supernormal phase (see p. 577 below). Certain differences in its rate of action and degree of reversibility as compared with hydrochloric and acetic acids are probably due to its greater penetrating power.

Cooper (1923) concluded that in partially asphyxiated nerve the rate of recovery from the refractory period is unchanged, but she employed Lucas' (1913) method of measurement which Kato (1926) has shown to be fallacious. Her results, interpreted in view of Kato's work, indicate a definite slowing of recovery, but this may be a secondary effect of accumulating products of metabolism; and it remains uncertain whether oxygen enters into the immediate recovery from the refractory phase after every impulse, or whether it merely serves to maintain an energy reservoir at a proper level. This latter *rôle* is suggested by Hill's observation of delayed heat production continuing for as long as ten minutes after a brief period of stimulation; and the phenomenon of delayed heat and the broad analogy with muscle metabolism support Gottschalk's suggestion that the primary release of

energy is anaerobic. The analogy with muscle is somewhat strengthened by observations of Thunberg (1923) on the enzymes present in peripheral nerve. He found that minced nerve would decolorize methylene blue, and that, if this power were reduced by washing, it could be largely restored by the addition of lactic acid, glutamic acid, and other substances which appear in the intermediary metabolism of muscle. If these effects were not due to the connective tissue present in the nerve trunk, it would seem that the nerve fiber contains enzymes capable of dehydrogenating (i.e., "oxidizing") these substances.

Thörner (1922 a, b, and c; 1924) has investigated the effects on the threshold of excitation of oxygen lack and other unfavorable conditions such as excessive heat. Thörner, Woronzow (1925), and others also describe modifications of the phenomena of electrotonus. Detailed discussion of these topics are beyond the scope of the present review, but Thörner's observations, in spite of possible technical criticisms, support the view that the resting nerve like other cells is in a state of dynamic equilibrium for the maintenance of which oxygen is essential.

Evidence from temperature effects. Temperature coefficients have frequently been invoked to decide whether nerve conduction is a chemical or a physical process; but although it is true in general that at ordinary temperatures the rates of "irreversible" chemical processes are doubled or trebled by a ten degree rise in temperature while physical processes are relatively little affected, some caution must be observed in drawing conclusions from data of this type. Hill (1921) has emphasized that the temperature coefficient of a complex process such as nerve conduction is the coefficient of the slowest or limiting step which may be otherwise relatively unimportant. To illustrate this, he determined the coefficient for the velocity of burning of gunpowder packed in an iron trough, and finds it to be about 1.01. The very low Q_{10} is of no particular significance in this case, since the heat of the reaction itself alters to a large and uncertain degree the temperature at which the process is occurring; but probably the limiting factor is the physical process of spread of heat along the trough, not the chemical process of combustion.

In table 2 are collected typical temperature coefficients (determined from data obtained since 1906) of various nerve functions. Certain comparable data on muscle and the neuromyal junction are also included. It will be seen that the temperature coefficients for the velocity of the nerve impulse are nearly all of the "chemical" type. Referring to our previous analysis (p. 555) of the conduction process, this merely

indicates that somewhere, perhaps in 1, the development of energy by the characteristic reaction, or 2, the spread of energy, or else in 3, the excitation process, is interposed a chemical reaction. The low coefficient for the summation interval indicates that here a "physical" process, probably the diffusion of ions, is the limiting factor.

Most of the data available depend on observations made at a few widely separated temperatures. Crozier (1924a, 1924b) and others (see bibliographies in Crozier's papers) have shown clearly that the temperature coefficient for a biological process is not necessarily the same throughout the physiological range but often changes abruptly at definite "critical temperatures". The exact value of the ordinary Q_{10} index for such a process may depend largely on the choice of experimental temperatures with respect to these critical points. Perhaps part of the divergence in the coefficients given in Table 2 is due to the difference in temperature intervals. The "critical increment" (cf. Crozier et al., loc. cit.) is a more satisfactory index for comparison, and has been included in the table where the observations are numerous enough to allow it to be calculated. Broemser's (1921) unique finding of a "physical" coefficient for conduction velocity depends on the observation that the velocity of conduction tends to return to normal when the temperature is maintained for a considerable time at the new level. This suggests some sort of adaptation to the new conditions, which, if substantiated, might even explain such gross divergences as that between Fulton and Samojloff on neuromyal delay.

THE ELECTRIC DISTURBANCE. The only index by which we may follow the course of the local reaction in the nerve is the transient alteration of electrical potential associated with it. This is termed the "action potential" by Erlanger and Gasser (1924) when recorded by an instrument which draws no current, corresponding to the familiar "action current" for the current which flows through a galvanometer. Both of these are different from the "local bioelectric currents" (Lillie, 1922, 1923) which flow in the tissue itself as a result of the potential differences. Gasser, Erlanger, and Bishop[3] (1922, 1926c) and Davis and Brunswick (1926) have emphasized the impossibility of inferring the magnitude of the potential change in the nerve fiber from observations of the action current, since the circuit through the instrument

[3] In referring to the papers of these authors they will for brevity be treated as a single series, although the order of the authors' names varies from paper to paper. But, in the bibliography, some will appear under Erlanger, some under Gasser, some under Bishop.

TABLE 2

Temperature coefficients of nerve function

	YEAR	ANIMAL	TISSUE	TEMPERATURE RANGE	Q_{10}	CRITICAL INCREMENT* (μ)
Velocity of conduction						
				°C.		
Maxwell	1907	Ariolimax	Pedal nerve	0–26	1.78	8,000; (18,200?)†
Snyder	1908	Frog	Sciatic nerve	0–30	1.6–3.0	12,900
Lucas	1908	Frog	Sciatic nerve	8.5–18.5	1.8	
Ganter	1912	Frog	Sciatic nerve	0–30	1.75	8,600; 12,200‡
Broemser	1921	Frog	Sciatic nerve	8–32	1.02§	
Samojloff	1925a	Frog	Sciatic nerve	0–20	1.72	8,300
Rosenberg and Sugimoto	1925	Frog	Sciatic nerve	12.5–26	1.56	
Rosenberg and Sugimoto	1925	Frog	Sciatic nerve	Below 12.5	>1.6	
Woolley	1908	Frog	Sartorius muscle	5–15	2.01	
Woolley	1908	Frog	Sartorius muscle	10–20	1.79	
Lucas	1909	Frog	Sartorius muscle	8–18	1.5	
Samojloff	1925a	Frog	Neuromyal junction	0–20	2.6¶	15,500¶
Fulton	1926	Frog	Neuromyal junction	4–24	1.4–1.85	Inconstant
Rate of rise of action current						
Lucas	1909	Frog	Sartorius muscle	8–18	1.5	
Bishop and Erlanger	1926f	Frog	Sciatic nerve	Δ		

			Recovery (relative refractory period)			
Adrian	1914c	Frog	Sciatic nerve	8–18	3.0	
			Summation interval for subminimal stimuli			
Lucas	1910a	Frog	Sciatic nerve	8–18	1.3	

This table contains representative data obtained since 1906. Certain figures on muscle and on the neuromyal junction are included for comparison.

* The writer is indebted to Prof. W. J. Crozier (1926) for determining these increments from the published data.

† Certain series of measurements.

‡ Frogs long in captivity.

§ Inferred from erratic data. See text, p. 583.

¶ These values are not corrected for the time required for conduction in the intramuscular portion of the nerve.

Δ The coefficient is less for the rising phase than for the falling phase, but the data are too meager to warrant calculating the Q_{10}.

is shunted to an unknown degree by idle tissue and fluid. Furthermore, the ordinary string galvanometer moves much too slowly to give even an approximate idea of the time course of the disturbance in medullated nerve.

By far the best data on the electric disturbance are those of Erlanger, Gasser and Bishop (1922, 1924, 1925, 1926), recorded by means of a cathode ray (Braun tube) oscillograph. The great advantage of this device is that, owing to its freedom from mechanical inertia, it yields time records of action potentials with a maximum error of 0.02 σ (1926c) (1 σ = 0.001 sec.).

The compound nature of the action current and the velocity of conduction in various fiber groups. With the cathode ray oscillograph it was soon found that the exact shape of the action potential record depends not only on the potential change in each fiber but also to a large degree on how perfectly the disturbances in various fibers are synchronized. Records from the phrenic of the dog (1924) show a 50 per cent increase in time to maximum and total duration and a reduction of 33 per cent in amplitude as a result of traveling 95 mm. The total energy of the disturbance, however, remains constant (1926c). This indicates a considerable range of velocities among the fibers of even so homogenous a nerve as the phrenic. In a mixed nerve, such as the bull-frog's sciatic, the differences in velocity are even more pronounced, so that definite waves appear in the falling branch of the action potential record. These secondary waves represent groups of fibers of relatively low conduction velocity in which the impulses are lagging farther and farther behind the main group. As a rule, three and sometimes four such groups may be identified; α, the largest and fastest; then β, γ, and δ in order of both size and velocity. It is possible to measure the velocity of each group with fair accuracy, the limiting factor being the differences among individual fibers in a group and not instrumental error. In fact there is rarely a sharp separation between groups. We should think rather of peaks in a distribution curve. (Cf. 1926g.) Typical data are collected in Table 3. The figures are for the fastest fibers in each group. The fastest fiber in a group may be 50 per cent or more faster than the slowest.

As a result of extensive and ingenious tests Erlanger, Gasser and Bishop (1924, 1925, 1926) leave no doubt as to the explanation just outlined for the irregularities seen in the compound action potential record. They show that the thresholds differ for the various groups, being lowest for α, higher for β, and so on. By carefully grading the

stimulus they can obtain a pure α wave. A second stimulus, a little stronger, falling in the absolute refractory period of α will give a pure β wave. The time relations, magnitude, and velocity of this pure wave agree closely with those inferred from the compound wave. Further evidence is found in the different durations of the refractory period. The absolute refractory phase in a given fiber lasts 1.20 σ, 1.68 σ, and 3.13 σ for the fastest of the α, β, and γ fibers respectively in the green frog at room temperature, and the relative refractory

TABLE 3

Velocity of conduction

PREPARATION	TEMPERATURE	VELOCITY, METERS PER SECOND			
		α	β	γ	δ
	°C.				
Bull frog sciatic	24.4	47.3	28.7	19.0	13.6
Bull frog sciatic	22.3	43.8	27.0	16.2	
Bull frog sciatic	21.0	41.3	25.6		
Green frog sciatic	23.3	33.3	17.0	9.4 (?)	
Dog saphenous	38.0	83.3	57.8		
Dog tibial	38.0	80.4			
Dog vagus	38.0	53.6			
Dog phrenic	38.7	52.9			
Dog splanchnic	37.0	9.6			

Selected from the data of Erlanger, Gasser and Bishop (1924). Measurements were made of the time of arrival of the foot of the α wave, representing the fastest fibers, and the lag behind this of the β, γ, and δ waves. Measurements are from the start of the stimulus, uncorrected for utilization time or possible spread of stimulus. Where no figures are given, the secondary waves were not distinct enough to measure. They are never seen in the phrenic nerve.

For other data on velocity by less satisfactory methods consult particularly the references cited in connection with the temperature coefficients, and also Kato's monographs.

periods bear the same ratio. In the phrenic of the dog the absolute refractory phase may last only about 0.6 σ (1925c). With equal clearness and conclusiveness and a wealth of experimental evidence, the change in shape of the α wave itself is shown to depend on velocity differences. In fact there is often no clear separation between the slowest of the α group and the fastest of the β.

Lapicque and Legendre in 1913 had noted a correlation between diameter of the nerve fiber and its "chronaxie", i.e., the index of speed of the excitation process. In the frog the chronaxie is inversely pro-

portional to the cross-sectional area of the fiber. Lapicque, Gasser and Desoile (1925) have pointed out that relative uniformity in size of fiber characterizes the phrenic nerve which shows no secondary waves in its action potential, while the sciatic is made up of fibers varying widely in diameter. Gasser and Erlanger (1926g) have studied this relationship in detail, and find a linear relationship between the velocity of conduction and the diameter of the fiber. Using their best data for the duration and relative magnitude of the potential wave in each fiber they are able on this hypothesis to construct theoretical action potential waves which closely resemble the compound waves actually recorded. We may say quite confidently, as a first approximation, that the velocity in a fiber is determined by the diameter. This relation holds within a given species, and the rather scanty data available suggest that it may also apply between green frog and bull-frog, and even (assuming a temperature coefficient of 1.7) between frog and dog.

The same investigators (1926d) find that all four waves enter the spinal cord by the dorsal root, and that only α appears in the motor root. From this it is obvious that all experiments on motor nerve stimulation in which muscular response serves as the index of activity give information about the α group only. The phrenic nerve shows only the α wave, and it is inferred from this that the sensory α group in general includes the fibers for muscle sense. In a preliminary report (1925a) they state that sensory α in hind limb nerves evokes motor hind limb reflexes and depressor reflexes, while stimuli strong enough to initiate the β wave may elicit respiratory and pressor reflexes, although the latter may appear only with δ.

The axon potential wave. This term (Erlanger, Bishop, and Gasser, 1926c) is convenient to designate the electric disturbance in the individual fiber. Its magnitude and time course are what we need in order to gain an insight into the nature of the local response; and to approximate it, we obviously must record the action current as close to the stimulus as possible in order to minimize temporal dispersion.

Action potentials recorded within 10 mm. of stimulus or even at the stimulating electrode (1926b and c) show a rapid rise to maximum, completed in 0.4 to 0.5 σ in amphibian nerves at room temperature, and a slower fall ending so gradually as to render precise time measurements difficult. The order of magnitude of the total duration is 1.5 to 2.0 σ. Both time to maximum and total duration are proportionately shorter in the dog's phrenic at 38°C., being roughly 0.3 σ and 0.7

to 1.0 σ respectively. The action wave starts within 0.05 σ of the very brief induction shock used as stimulus, but the "utilization period" apparently varies somewhat from fiber to fiber (cf. Gildemeister, 1913). On the other hand, the time relationships of the electric disturbances in the various fiber groups (1924, 1926c) are practically identical with one another. The height of the β, γ, and δ potential waves are much less than that of α, but this may be quite as well due to a smaller number or total cross-sectional area of fibers in these groups as to a lower potential in each. The commencement of the rise in potential is more abrupt the more nearly perfect the synchronization, and, in the semi-logarithmic coördinates in which the Braun tube records appear, both rise and fall are often practically linear over most of their length.

On the basis of this shape of the potential wave and the differential effects of temperature changes and polarization, Bishop and Erlanger (1925b, 1926f) tentatively suggest that we are dealing with a "discharge" reaction running more or less completely to a limit and a second slower "recovery" reaction which checks the discharge and restores the resting equilibrium. Apparently the position of the equilibrium may be shifted by various influences, but the whole question of electrotonus and polarization is still so confused and speculative that adequate treatment is impossible in the present review. Consideration of the "positive after-variation" and its possible significance must also be omitted. Bishop and Erlanger (1926f) analyze the effects of polarization most completely, but the papers of Verzár (1924, 1926), Samojloff (1925b), Garten (1910a), Sochor (1912), Tigerstedt and Donner (1913), Vészi (1912), Vassilieff (1922), Woronzow (1924) and others may also be consulted.

The source of energy of the action current. In spite of its obvious importance a complete treatment of this subject is likewise beyond the scope of the present article. The most generally accepted theory, dating back to Ostwald (1890) and Bernstein (1902, 1913) seeks to identify the origin of the action current with that of the current of injury. Differences in concentration of electrolytes inside and outside of the cell constitute the immediate store of energy. The differences are maintained by a semipermeable membrane, which normally allows cations to pass through but restrains the anions and thus becomes polarized. Destruction of the membrane allows depolarization and thus gives rise to the current of injury, and a transient increase in permeability gives rise to an "action current". Lillie has incorporated these ideas in his theory of conduction (1922, 1923), and reviews the

question of origin of bioelectric potentials. It is implicit in such a theory that any equalizations of concentration resulting from "activity" are subsequently compensated by metabolic activity of the cell (Adrian, 1920). Pauli and Matula (1916) oppose this conception of preëxisting potentials in tissue and their observations on the effects of temperature on the injury current do not substantiate Bernstein's familiar conclusions that the injury current is roughly proportional to the absolute temperature of the uninjured tissue, and that the temperature of the injured region is immaterial. The phenomena are evidently more complex than the original "membrane theory" indicates. As to the nature of a semipermeable membrane, Beutner's work (1913, 1920) with oily solvents which allow the passage of organic ions but restrain inorganic ions is suggestive, particularly in connection with Clowes' (1916) hypothesis that the membrane consists of a water-lipoid emulsion capable of phase reversal. Although both workers are able to construct interesting models their ideas cannot as yet be accepted unreservedly. Rohonyi (1922) criticizes Beutner, and suggests that a better analogy for the cell membrane is that of a "precipitation membrane" which essentially interposes a layer of electrolyte-free water between solutions of different concentrations. But he does not show that such models as he constructs would give a summated potential when connected in series, which Brünings (1903) has shown possible with muscle cells injured at one end. Mond (1924) shows that protein gels may be substituted for Beutner's lipoid phase, giving effects which he considers an even better parallel to biological phenomena. The relation of the potentials of such cells to the hydrogen ion concentration of the solution is of some interest in connection with the work of Andrus (1924) and Drury and Andrus (1924) showing that a moderate increase in acidity of the perfusate depresses and alkalinity increases the action current from auricular muscle. Lillie (1923, p. 301 ff.) emphasizes that the "membrane" theory is not necessarily a modified diffusion theory, and makes the suggestion that oxidation and reduction potentials and adsorption potentials may be concerned, but does not develop the idea at any length. Bishop and Erlanger (1926e) warn against any oversimplified and static explanation, and regard both action and resting potentials as the resultants of balanced reactions.

There is no agreement as to the morphological location of the postulated membranes, and many workers are deliberately noncommittal on this point. It is usually assumed to be the outer "cell membrane" of the nerve fiber. We must not think of this membrane as a mere passive

sieve, but as the boundary of active protoplasm. (Cf. Lillie, 1923; Chambers, 1924; Lapicque, 1925a.) Some workers (e.g., Bethe, 1916; Göthlin, 1917; Boeke, 1921; Brinkman and Szent-Györgyi, 1923; and others, particularly among the older investigators) specifically suggest the boundary between neurofibrils and axoplasm as the seat of the fundamental reaction. On the other hand, many histologists (Mott, 1912; Marinesco, 1912; Chambers, 1926; Matsumoto, 1920; Lewis and Lewis, 1924), using methods of vital staining, dark-field illumination, and microdissection, fail to find fibrils in living nerve cells and regard them as products of post-mortem coagulation.

EXCITATION. Before considering the mechanism of the spread of energy from point to point on the nerve fiber it will be profitable to consider the process of excitation which that energy is supposed to induce at successive points, and also the recovery process following response.

Nearly all experimental work on excitation has employed electricity as the stimulus. We shall not attempt a detailed review even of recent work, but merely mention a few salient points. The central assumption of the familiar Nernst theory is the existence of a membrane impermeable to ions, at which polarization would occur. Nernst (1908) deduced mathematical relations between strength and duration of a constant current just adequate to excite. The formula failed to fit the experimental facts for currents of relatively long duration. Hill added the assumptions 1, that the opposite ends of the cell are at a finite distance from one another; 2, that it is accumulation of cations which stimulates, and 3, that the accumulation must occur at a certain minimal rate. Even this formula has not proved entirely satisfactory. Eucken and Miura (1911) suggested adding another term, a function of intensity of current, to obtain agreement for very strong currents. Umrath (1926) and Jinnaka and Azuma (1922a and b) found marked variations in the constants of the equation as calculated from various parts of a given strength-duration curve, and the latter authors suggested instead an empirical hyperbolic formula. Jinnaka and Azuma (1922a and b) and Davis (1923) found that the values of the constants varied (in the case of muscle) with the type of electrode. (Cf. also Verryp, 1924.) Jinnaka and Azuma (1922a) and Gildemeister (1913) point out that one source of error in such experiments is deformation of the current by the self-induction or polarization of the tissue; and Gildemeister (1925) even suggests that this phenomenon may be an important factor in preventing the excitation of one fiber by the action current of its

neighbor. Vogel (1925) shows that living tissue between nerve and electrode may markedly prolong the apparent chronaxie of the nerve. We cite these cases of uncertainty and disagreement, which are only a few of many in the literature, merely to show that the theory of electrical excitation is not yet in a satisfactory state. The hope expressed by Lucus (1912) that study of the process of excitation would reveal the nature of the propagated disturbance has not yet been realized. Nernst's assumption of a polarization phenomenon has not been overthrown, but it seems probable that an electric current can affect excitable tissue in more ways than one, causing a readjustment of equilibria. Apparently Nernst and Hill both recognized this, but could not include it adequately in their treatments. In spite of the efforts of Nernst (1908), Lapicque (1907, 1908a, 1909a), Hill (1910), Lasareff (1910), and others we have as yet no satisfactory theoretical and mathematical description of the phenomenon. Recently Lapicque (1925b), on the basis of experiments of M. and Mme. Chauchard (1925) on the summation of stimuli in the foot of the snail, has expressed doubt as to whether polarization can be considered the fundamental step in the excitation process, and he embarks on a new mathematical treatment of an empirical character. The experiments are of interest, since the authors obtain stimulation with interrupted current (charging a condenser) of a lower voltage than the threshold for a constant current. Unfortunately the experiments are not clean cut, since the relatively slow discharges of the condenser which also pass through the tissue are disregarded, and the tissue itself is complex. Lapicque's inference from their result is that polarization opposes rather than aids excitation, and he elsewhere (1925c) expresses the view that the phenomena of electrotonus are the consequences of polarization.

Although exact theoretical description of the relation between duration and minimal intensity of stimulation is as yet impossible, nevertheless the relation is sufficiently alike for all tissues to justify comparisons of speed of the excitatory process based on the assumption of similar curves. This is most conveniently done in terms of some more or less arbitrary index, such as Gildemeister's *Hauptnutzzeit* (1913), v. Kries's *Speicherungsvermögens* (1919), or Lapicque's *Chronaxie* (1909b). (Cf. Fulton, 1924, for a brief review of Lapicque's work on chronaxie, and Bourguignon (1923) for its clinical application to man.) The two latter are practically identical although defined very differently. The chronaxie is the least *time* required for excitation by a current just double the threshold strength for a constant current of

relatively long duration. For medullated amphibian nerves it is of the order of 0.2 to 1.0 σ. It shows interesting correlations, as already noted, with size of fiber, velocity of conduction, and also with the speed of response of the muscle it serves. Lapicque and Legendre (1922) follow the diminution in chronaxie induced by various anesthetics, and show that it runs parallel to the development of local swellings in the myelin sheath. Lapicque believes that a motor nerve and its muscle have the same chronaxie and that conduction of the impulse from nerve to muscle is possible only when the chronaxie of one is not more than double that of the other (1908b). He explains the action of curare (and other drugs (1913)) as abolishing the normal "isochronism"[4]. This theory is severely criticized by Watts (1924) who is unable to confirm the exact relations described by Lapicque. (Cf. also Vogel, 1925.)

There is considerable diversity of opinion as to the intimate mechanism of excitation. Mathews in 1902 attributed a specific excitatory effect to the hydroxyl ion. Bethe (1916) on the other hand ascribed a similar rôle to the hydrogen ion. Andrus (1924) stresses the difference in pH between inside and outside of the cell in determining excitability. Lillie (1911) postulated a mutual interdependence between the polarization and the permeability of the membrane, and more recently (1923) suggests that an electrolysis of the membrane causes the increase in permeability. Broemser (1925) considers osmotic changes of primary importance. Ebbecke (1924) suggests that a relatively slow adjustment, presumably a change in the condition of the membrane, may act like a constant excitation while change continues, and lead eventually to electrotonus when complete. The author bases his ideas on the phenomenon of repetitive discharge to a constant stimulus, which he believes to be a normal response of nerve trunks as well as sensory end-organs. Adrian and Zotterman (1926) express a similar idea of slow adjustment in their discussion of the organs of muscle sense. Ishikawa (1924) believes that multiple discharge in response to a single stimulus is normal for nerve, and classes "rhythm making ability" or "automaty" as one of the fundamental phenomena of life. Some of his experiments are of interest, but in themselves are not sufficient

[4] While skeletal muscle is supposed to have normally the same chronaxie as its motor nerve the same relation does not exist in the case of smooth muscle and non-medullated nerve. Here "heterochronism" is normal, and associated with this is the necessity for a summation of nerve impulses to initiate a contraction. (Lapicque, 1912.)

basis for the extensive theory which he erects. It may be noted that he observes "automaty" in nerve most frequently under distinctly abnormal conditions. For his theory he postulates a "proto-excitation" as distinct from "accompanying excitation", but the concept is vague and impossible to translate into physico-chemical terms.

The excitatory process gives us no certain clue as to the nature of the impulse, but it is undoubtedly one element in the cycle of conduction, and its study has given us important facts by which we may test certain theories of nerve function.

THE REFRACTORY PERIOD AND THE RECOVERY PROCESS. Any theory of nerve function must explain the refractory period. It is a serious stumbling block for purely physical theories of wave motion (e.g., Sutherland, 1905), or vague "resonance" theories as proposed by Weiss (1924, 1926). (Cf. Detwiler, 1925, and Forbes, 1926, for discussion of this theory.) For an interval of 0.6 to 3.0 σ (see p. 569) following the passage of an impulse the nerve will not respond again to a stimulus of any strength. This is the absolute refractory period as defined by Forbes, Ray and Griffith (1923). Then, during the relative refractory period, roughly 10 σ in length, excitability progressively returns.

Tait in 1910 advanced the hypothesis that the absolute refractory phase is associated with the rising potential of the electric disturbance and the relative refractory period with the return to normal. Adrian (1921) showed that, while this is true in the frog's sartorius at room temperature, it is an accidental relationship which vanishes at low temperatures. The recovery process has a much greater temperature coefficient than the electric disturbance (see table 2, p. 566). The durations do not correspond at any temperature in nerve (cf. also Erlanger, Bishop and Gasser, 1926c), or in cardiac muscle. Lillie (1920, 1925) finds a similar dissociation in the case of his iron wire model, and it seems clear that the "recovery process" of Bishop and Erlanger (1925b, 1926f) which checks the electrical discharge is quite distinct from the "recovery process" underlying the relative refractory period. As Lillie suggests, the first may be a stabilizing reaction, while the second brings the system to a state of metastable equilibrium.

Gasser and Erlanger (1925c) have studied the electric response and the velocity of conduction during the relative refractory period. There is no doubt that the electric response and velocity of conduction are reduced, more in the earlier stages of recovery than later. The distortions due to temporal dispersion make it impossible to judge with

certainty whether the electric response becomes fully normal before full return of excitability or not, but this is quite possible. The velocity of conduction is apparently not fully normal unless the second impulse follows the first by several thousandths of a second, although it is already very nearly normal with an interval of 1.5 σ in the case of the dog's phrenic. Since the velocity of the second impulse is less than that of the first, it is obvious that the second will lag farther and farther behind the first and that, as it lags, it will travel in more and more completely recovered nerve. Thus, in spite of lagging behind the first, its absolute velocity and action potential will progressively increase. If the second impulse is set up early in the refractory period, the absolute velocity is very low indeed, and the resulting lag accounts completely for the familiar delay of the second impulse (Gotch, 1910; Lucas, 1910b) without the necessity of postulating a persistence of the local excitatory process as suggested by Forbes, Ray and Griffith (1923).

The observation of Kato (1926) that the refractory period following a subnormal impulse is as long as that following a normal one has already been discussed (p. 552), and likewise his conclusion, opposed to that of Lucas, that the recovery process is prolonged by narcotics, asphyxia, and the like. Beritoff (1923) believes the recovery process, as measured by the size of the electric response, and also by recovery of excitability, to be prolonged by cocaine; and he explains the so-called "Wedensky inhibition" on this basis.

We may call attention once more to the several distinct phenomena to which the term "recovery process" is applied. As a rule it means "recovery of excitability" (Adrian, 1920; Lucas, 1917) as measured by the refractory period. Logically distinct from this, although the two may be interdependent, is the "recovery of response", measured by the return of the electric response to its normal size. Then there is the "recovery process" of Bishop and Erlanger which is supposed to check the development of the action potential and restore the tissue to its original electrical state.

The supernormal phase. Adrian and Lucas (1912) reported a stage of supernormal excitability following the refractory period, and also showed that, if two impulses were properly spaced, the second might pass a region of partial narcosis although the first fails. From this they infer a "supernormal phase in conductivity". More recently Adrian (1920) has demonstrated that the supernormal phases of both excitability and conductivity are absent unless the perfusing medium is more acid than pH 7. The excitability in acid solution returns

temporarily to the resting value found in neutral surroundings and then gradually falls to a somewhat lower level, apparently giving a supernormal phase. In alkaline solution the recovery is slower and the excitability reaches a value somewhat below that in neutral solution without overshooting. The apparent contradiction between this result and that of Andrus (1924) and Hemingway (1926) may be due in part to the different tissues and means of stimulation, and in part to the greater alkalinity of Adrian's solutions (pH = 8.3 and 9.5).

On the basis of observations on cardiac muscle Adrian (1921) concludes that the electric response in the supernormal phase is normal in size. By analogy to the relative refractory period we might have expected an increase in both action potential and velocity of conduction; but there is no necessary relation between electric response and excitability. Lower threshold, however, should certainly increase velocity of conduction, *ceteris paribus*. Cooper (1925) reports no increase in velocity in the supernormal phase, but in these experiments she finds a normal velocity for the first impulse, although the surrounding fluid is distinctly acid (pH 5), which is contrary to the results of Drury and Andrus (1924), Kato, Shizume, and Maki (1921), and Kato (1924b). Cooper elsewhere (1924) had reported evidence for depressed activity and "decremental" conduction in nerves which also showed the supernormal phase, all of these effects being induced by acid solutions.

Lucas (1917) attempted to explain summation of successive impulses at a neuromyal junction or synapse on the basis of the supernormal phase in recovery and decremental conduction, but it is not clear how these principles alone would cause summation unless we postulate also a supernormal phase of response. Satisfactory evidence for such a phase is apparently lacking. Adrian (1920) and Forbes (1922) have discussed the difficulties imposed on Lucas' explanation by the dependence of the supernormal phase on the acidity of the surrounding medium, and conclude that they are not insuperable. If, as Kato believes, conduction with a decrement does not occur, another difficulty appears; but the "transitional decrement" of Davis, Forbes, Brunswick, and Hopkins (see p. 555) makes the difficulty less serious for a structure as small as a synapse. Even so, something akin to supernormal response or a "treppe" seems essential to explain the phenomenon of summation in conduction.

The Wedensky inhibition. The converse effect to summation in conduction, in which the first impulse of a properly timed series will

pass a region of depression while the second and subsequent impulses fail, is known as the Wedensky inhibition and has been widely invoked (Forbes, 1922; Adrian, 1924; Veach, 1925) in explanation of both central and peripheral inhibition. Lucas (1911) and Adrian (1913) showed how it depended on each impulse of a series travelling in the relative refractory period of a predecessor, but they explained the mechanism of extinction in terms of decrement. Kato (1926) shows that the phenomenon is quite independent of decrement. Developing the suggestion of Forbes, Ray, and Griffith (1923, p. 605) it seems probable that extinction occurs because the subnormal impulses in the relative refractory phase as they reach the narcotized region do not liberate energy enough to excite the narcotized nerve with its raised threshold, although the response of fully recovered nerve liberates, even in the narcotized region, energy enough to maintain the progressive excitation. The possible importance of this phenomenon in the central nervous system is critically discussed by Fulton (1926) and does not concern us here.

THEORIES OF THE "SPREAD" OF ENERGY AND THE MECHANISM OF CONDUCTION. The question of the manner of spread of energy from the region of its liberation to the region where it excites a fresh liberation of energy has been one of speculation rather than experiment. The commonest assumption has been that the energy is electrical. Cremer has developed this idea with the further assumption that the nerve is a so-called *Kernleiter* or core conductor; i.e., two parallel conductors separated by a polarizable interface. A potential difference applied to the conductors will give rise to a wave of polarization which travels along the interface. He designates one conductor as "core", the other as "sheath", and derives the following formula (Cremer, 1909) for velocity:

$$V = \sqrt{\frac{W_K}{W_H} \cdot \frac{E_{act}}{\tau} \cdot \frac{M}{E_R}}$$

The terms under the radical signify, briefly, 1, the ratio of longitudinal electrical resistance of core to that of sheath, 2; the rate of development of the action potential, and 3, the electrical energy necessary to stimulate. Numerous workers in his laboratory (cf. Cremer's Beiträge, 1922–24; Plaut, 1923; Rosenberg and Schnauder, 1923) have endeavored to establish the values of the various constants, and with their approximations the formula gives a fair check with experiment in the case of frog's nerve, a rapidly conducting tissue, and nerve from *Anodonta*, a "slow" tissue (Cremer, 1924).

Lillie follows a similar idea in less definite form and likewise derives a velocity formula: $V = K\frac{s}{t}$. Here K is a constant, s is the maximal distance at which the spreading electrical potential reaches a stimulating value, and t is the time required for it to rise to its maximum. In support of this theory, Lillie (1914) has emphasized the broad correlation which exists between the velocity of conduction and the rate of rise of the action current in various tissues. He also determined s experimentally as approximately 3 cm., but it is evident from his method that the exact mode of electrical potential spread was not carefully considered. Erlanger, Bishop and Gasser (1926c) show that Lillie's figure is impossibly large. They determine the position of the active region by the commencement of the refractory period and show that a rise in potential sufficient to be detected by their oscillograph does not extend in advance of it more than 2.9 mm. at most,—a figure scarcely greater than their experimental error. The time required for the impulse to travel this distance is only 0.07 σ. If the electrical theory is correct, therefore, it must be assumed that a potential of not more than one-fifth of that of the action current, rising to its maximum in not over 0.07 σ, must suffice to excite each successive point along the nerve (cf. also Bishop, Erlanger and Gasser, 1925b). When we recall that the recorded maximum action potential is of the order of only 50 millivolts, and that the chronaxie of nerve is about 0.3 σ, this would seem to amount to an impossibility; but we must note that we have as yet but little knowledge of the fraction of the total bioelectric potential which reaches our recording instrument, or the fraction of our stimulating current which is utilized in excitation (cf. p. 565). Also, as pointed out on page 573, chronaxie measurements may be highly deceptive. The utilization period for a brief induction shock applied to nerve is not over 0.05 σ (Erlanger, Bishop and Gasser, 1926b). Such considerations may eventually lead to a solution of the difficulty.[5]

The present writer believes that an electrical theory along the general lines followed by Cremer and Lillie is the most promising working hypothesis. Qualitatively it meets the requirements which the all-or-none law and our knowledge of nerve metabolism impose on any

[5] Lillie (1925) also suggests that the relatively high velocity of transmission in medullated nerve may depend on the structure of the myelin sheath, with its periodic interruptions at the nodes of Ranvier. An iron-wire model enclosed in a continuous glass tube shows a much slower transmission than when the tube is broken up into series of discontinuous segments.

theory. Many correlations, such as those between action current and conduction velocity, find a natural place in it. The objections to it are quantitative, and we may hope that with the improvement of technique they may be shown to rest on inadequate data.

In the above theories of conduction, the spread of potential is regarded as essentially similar to that in a poorly insulated power transmission line, and variations of velocity with changes in the electrical resistance of the surrounding medium are invoked in support of the theory (Pond, 1921; Lillie, 1925). Observations of Mayer (1917) and of Broemser (1920) may also be interpreted in this way although Mayer emphasizes the importance of other factors, such as ionic ratios, and Broemser stresses osmotic pressure instead of electrical resistance.

Göthlin (1910) and Crehore and Williams (1913) suggested the analogy of a "cable" conductor in which the velocity of a transient potential wave is determined by the resistance and the distributed capacity of the conductor. Göthlin developed this theory at some length, and made numerous measurements of the physical constants of nerve tissue. His calculations led to the conclusion that the nerve fiber must supply energy by some chemical reaction in order to maintain the amplitude and velocity of the potential wave, so that his theory differs essentially from Cremer's and Lillie's only as to the factor limiting the velocity of transmission. According to his theory the velocity should be inversely proportional to the longitudinal resistance, and therefore proportional to cross-sectional area, whereas actually it is proportional to the diameter (cf. Gasser and Erlanger, 1926g). Göthlin's (1917) assumption of a process "running around the ring of Nissl bodies in the nerve cell" to account for rhythmic discharge from the central nervous system would seem to be gratuitous.

A theory that nerve conduction depends on the spread of a capillary-active substance along interfacial boundaries was tentatively suggested by Brinkman and Szent-Györgyi (1923), but the further study of the phenomenon of surface spread by Cary and Rideal (1925) makes the idea seem far from plausible. Furthermore, to account for the known velocity of transmission it was necessary to assume a very high surface tension at the surface of the neurofibrils. The proponents of the theory suggested that the sharp curvature of the fibrils might cause such a tension; but this curvature is in a plane perpendicular to the direction of transmission. It is possible, however, that the passage of adrenin up a nerve, suggested by the observations of Lichtwitz (1908), which have been confirmed by Koppányi and Laughton (1925), may be by

some such mechanism if it actually occurs. The experiments of Wible (1924, 1925), apparently demonstrating a direct local action of veratrin on nerve, may possibly belong in this same class, although considerable evidence against this interpretation is presented. A discussion of the action of drugs on nerve, however, lies beyond the scope of the present review.

The chief interest of Brinkman and Szent-Györgyi's suggestion lies in a possible relation between nervous conduction and the humoral transmission of vagus and sympathetic effects from one organ or animal to another. This phenomenon has been extensively investigated by Loewi (1921) and many others. Brinkman and van Dam (1923) and Brinkman and v. d. Velde (1925) correlate these effects with changes in the surface tension of the blood. Such observations led Hamburger (1923) to voice the suggestion that nerve conduction, "humoral transmission", and the familiar hormonal effects might ultimately be reduced to a single type of activity. As yet, however, this is far from being the case, and an opposite trend is shown by investigators of the central nervous system (Sherrington, 1925; Fulton, 1926) who postulate types of activity in the cell bodies or synapses differing more or less fundamentally from the activity of the nerve trunk.

Beritoff (1924) reviews the subject of nerve and muscle activity and interprets excitation and response in terms of an "excitable substance". This is essentially a development of Verworn's "biogen" hypothesis, for the fundamental reaction of response is supposed to be the splitting of side-chains from living molecules. The place of energy in excitation and response is not recognized. A more specific objection to Beritoff's theory is that he believes the threshold of excitability and the magnitude of electric response to vary in parallel, and considers that both are expressions of the amount of "excitable substance" present. As we have seen above, this simple relationship does not always hold.

Broemser in 1920 proposed a theory, recently (1925) elaborated in great detail, in which the nerve impulse is regarded as a wave of osmotic pressure. Making a number of simplifying assumptions he formulates mathematically the conditions governing the propagation of such a wave. He recognizes that energy must be developed by the fiber to overcome the frictional losses of such a mechanism, and makes ingenious and complicated suggestions to this end, involving an acceleration of oxidation initiated by the differences in hydrogen-ion concentration which he concludes would result from rapid fluctuations in osmotic pressure. Electrical disturbances are also explained; but it is difficult

to see how a disturbance of the type of the familiar "wave of negativity" without a corresponding positive phase would result from his mechanism. He does not consider this difficulty specifically, all of his calculations being based on the assumption of a periodic cosinal disturbance. Elsewhere (1923a) he reports an analysis of string galvanometer records of the nerve action current which he interprets as a series of oscillations about a base line. He meets with some success certain criticisms of Brücke (1923) (Broemser, 1923b) but his observations are contrary to the general experience of other workers (cf. especially Erlanger and Gasser, 1924), and his inference that the impulse is in part "reflected from the cut end of the nerve", although consistent with his osmotic theory, quite disregards the phenomenon of the refractory phase.

As a test of the theory he calculates that the velocity should equal the square root of the osmotic pressure of the surrounding medium (and tissue) divided by the density of the fluid. The velocity in motor fibers of the frog's sciatic gives a surprisingly good agreement with this prediction (Broemser, 1920) and he finds also the predicted acceleration in more concentrated, and retardation in more dilute, solutions. Tanaka (1925) was unable to confirm these observations. (Cf. also Shōji, 1919.) Neither is any explanation apparent for the slower velocity in the smaller fibers of the sciatic nerve (cf. Gasser and Erlanger, 1926g). He states that the temperature coefficient of the velocity should be very small, corresponding to that of osmotic pressure. His experiments on this point (1921), in which he found an "adaptation" of the nerve to the new temperature, have been referred to. This he interprets as confirmation of his theory. Rosenberg and Sugimoto (1925) find no such adaptation, but Bishop and Erlanger (1926f) apparently encounter it in their study of action potentials. In a later communication (Broemser, 1926) he endeavors to explain the findings of Tanaka and of Rosenberg and Sugimoto on the basis of his elaborated theory, and maintains that, in such a complex series of reactions as he assumes, the temperature coefficient is not a crucial test. On the whole this theory, in spite of its ingenuity, seems to face much more serious difficulties than the electrical theory, but it may at least serve to stimulate valuable experimental work.

NON-MEDULLATED NERVE. In the foregoing discussions we have been dealing with medullated nerve, since it has been the object of investigation in most of the papers cited. There is no reason to believe that non-medullated nerve differs qualitatively in any essential respect. Its activities are slower, conduction time (Garten, 1910b; Schilf and

Hamdi, 1923; Yamada, 1923; Erlanger and Gasser, 1924), time to maximum of the action current (Garten, 1910), chronaxie and refractory period (Koenigs, 1915; Chauchard, 1922, 1924a and b; Tedeschi, 1924), and the least interval for summation of inadequate stimuli (Lapicque, 1912) all being much greater than in medullated nerve. Data on the electrical disturbance are comparatively scanty. The action current as recorded is usually distinctly smaller than from medullated nerve, perhaps due to a lower bioelectric potential or to high electrical resistance of the tissue, or else to a greater sensitivity of this type of nerve to trauma. Another feature which renders non-medullated nerve less available for precise investigation is that the tissues which it innervates are usually insensitive to a single impulse. The nerve itself responds to a single stimulus, but repeated stimulation is necessary to obtain the reaction of the muscle or gland which serves as the indicator of activity, and the added variable of frequency of stimulation must be controlled. Also the end-organ is often complex in structure, and it may show confusing spontaneous "tone" changes.

SUMMARY

The outstanding developments of the last decade in the study of the nerve impulse have been associated for the most part with improvements in technical procedure which have made possible analysis of a process involving a minimum of chemical action and energy liberation and occurring with extreme rapidity. The analysis of the electric disturbance, and the estimation of heat production and chemical metabolism are outstanding examples. We may justly hope for continued rapid progress along these lines. The exact nature of the nerve impulse still remains a mystery, but its general character as a progressive reaction, depending on energy liberation by the fiber itself, seems to be firmly established. Present evidence suggests that the form of energy concerned is electrical. The classical conception of "conduction with a decrement", and methods of investigation based upon it, have been seriously questioned, and the all-or-none law has been extended to include this apparent exception.

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THE REGULATION OF THE CORONARY CIRCULATION

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The mammalian heart rapidly fails as soon as the coronary blood flow is stopped (22). This alone is sufficient to show the importance of all factors concerned in the coronary circulation, a change in which may either lead to a disturbance in the action of the heart, or compensate a pre-existing pathological condition of the muscle itself, or may serve as a medium through which the activity of the heart can be influenced by therapeutic measures.

The experimental study of the coronary circulation has been conducted along three distinct lines, namely: 1, The investigation of the properties of coronary blood vessels; 2, the study of the effect of different phases of cardiac contraction upon the coronary blood flow, and 3, the study of the mechanical, chemical and nervous factors influencing the coronary circulation. In accordance with these three lines of enquiry, the experimental methods and the means of registration of coronary blood flow varied greatly in different researches. It is therefore not surprising that contradictory conclusions were often arrived at by different investigators. In reviewing the literature, a uniform conception as to the regulation of the coronary circulation can be formed only through a full understanding of the limitations of the methods employed. Moreover experiments have been conducted on a large number of different species of animals and since, at present, it is becoming more and more obvious that the properties of blood vessels in the different species are not always the same, a generalisation from results obtained with one type of animal can be made only with extreme caution.

EXPERIMENTAL METHODS EMPLOYED IN THE STUDY OF CORONARY CIRCULATION. Before discussing the different factors concerned in the coronary circulation, it is essential to consider first the methods which have been employed by various investigators. Experiments have mostly been performed on 1, isolated coronary blood vessels; 2, isolated hearts; 3, different forms of heart-lung preparations, and 4, the whole animal.

a. The isolated coronary blood vessels. Observations upon the contractions of isolated blood vessels were first made by Poisseuille (98) and later by John Hunter (48); Kühne (61) and Wertheim (131) elaborated the method and finally MacWilliam (76) used it in conjunction with graphic registration. Meyer (82) was the first to apply it to the coronary arteries. Strips or rings of an artery or vein are suspended in carbonate or borate Ringer, Locke, Tyrode solution or defibrinated blood and supplied with oxygen. The contraction of the ring is recorded by a suitable mechanical or optical lever allowing a magnification of 15 to 40 times. In order to increase the response and to avoid the necessity of using long levers, three or more rings can be connected in a chain (24). Rings of coronary blood vessels taken from the ox, horse, sheep, pig and man have been studied by this method. The method is of certain value so far as positive results are concerned, but, since the blood vessels are obviously studied under abnormal conditions, little importance can be attached to negative results. Furthermore, it is difficult to observe by this method the reactions of smaller arteries and impossible to draw any conclusion as to the reactions of arterials and capillaries. In this respect it should be borne in mind that smaller blood vessels and capillaries are, in many instances, known to react physiologically and pharmacologically in a different manner from the larger blood vessels. The observations made on isolated rings cannot therefore be applied to the whole coronary system without reservation.

b. Perfusion of non-beating strips of cardiac muscle. Porter (99) was the first to perfuse strips of heart muscle; in his experiments, however, the strips soon entered into spontaneous contractions. Elliott (33) perfused non-beating strips of the cat's ventricle. The method has been recently used by Subba Rau who found that after vital staining of the blood vessels with Janus green (Hoecht) 1/50,000, the absolute diameter of the coronary blood vessels including capillaries can easily be measured and they can be photographed. The same method can also be used for the study of coronary veins, especially if the perfusion is carried through the coronary sinus. Obviously the method can be used also for the study of the reactions of arterioles and capillaries.

c. Perfusion of the non-beating heart was used by several observers, chiefly by Wiggers (126), Kravkov (60) and more recently by Hammouda and Kinosita (45). The hearts are brought to a standstill by perfusion with non-oxygenated saline or Ringer solution, oxygenated saline, excess of potassium, strophanthine or arecoline. The best

method of perfusing isolated hearts is directly through the coronary arteries, though by taking precautions against leakage through the aortic valves, satisfactory results can also be obtained. It should be noted that changes in the coronary flow which are observed by the last two methods cannot always be attributed to vascular effects. This is due to the fact that the finer blood vessels are surrounded by the cardiac muscle which, though not contracting rhythmically, may under certain conditions enter a state of rigor or of a reversible contracture and so compress the intra-muscular vessels. It is important, therefore, to take full account of the state of the cardiac muscle even though it does not contract rhythmically (45). This can be done either by means of a lever attached to the apex, or by Cushny's myocardiograph, or, better still, by filling the left ventricle with fluid and measuring the size of the cavity. This is easily performed if the ventricle is brought into connection with a burette, the rise in the level of the fluid in the burette indicating the degree of contracture of the heart. Even so, there must be always taken into consideration the possibility of filling of the left ventricle through the Thebesian vessels.

d. Perfusion of the beating isolated hearts. This method was first used for the study of coronary circulation by Porter (99) and by Langendorff (62); these authors inserted a cannula into the aorta and measured the fluid emerging from the venae cavae. Schäfer (115) pointed out a source of error in this method arising from the imperfect closure of the aortic valves which allows a varying leakage of fluid into the left ventricle. For this reason, he modified the method by placing the cannula beyond the valves so that the fluid freely entered the ventricle, the escape of the fluid from the ventricle being prevented by tying off the pulmonary veins. The coronary arteries were supplied by the fluid passing along the sides of the cannula while the fluid in the ventricle was rhythmically ejected into the aorta. Wiggers finds Schäfer's modification unsatisfactory on the ground that the perfusion pressure alters considerably with changes in the strength of cardiac contractions. Furthermore he established that in perfusing the heart through the aorta, besides the variable and sometimes considerable leak through the valves the method is vitiated by the variation of the size of cardiac chambers which act as an intermediate reservoir between the heart veins and the registering apparatus. To obviate the first error Wiggers either drained the left ventricle or introduced the perfusing cannulae directly into the coronary arteries. To obviate the second error Wiggers worked on arrested hearts, though, as was pointed

out above, even the arrested heart may show considerable changes in the size of the chambers on account of a varying degree of contracture.

The methods of registration of the coronary circulation are based on measurements of the outflow of fluid from the isolated heart, or of the inflow into the heart. The simplest method of measuring the coronary outflow is to collect the fluid emerging from the right side of the heart; if the fluid is drained by means of a siphon placed in the right ventricle so as to keep its cavity always empty, this method gives reliable results provided that the leak through the valves in the case of aortic perfusion is drained off separately. Outflow can also be measured through a cannula inserted into the coronary sinus. This, however, leaves the Thebesian flow unrecorded. Brodie and Cullis (15) used an ingenious plethysmographic method by means of which constant records of the coronary outflow can be obtained; in the case of direct coronary perfusion this method is extremely accurate and is not vitiated by any change in the size of the cardiac cavities. Sassa (109) applied Frank and Ätzler's (7) method to measure the coronary inflow. The method briefly consists in counting the number of air-bubbles entering the constant pressure bottle which contains the perfusion fluid. Wiggers (126) measured the inflow by registration of changes in the diastolic pressure in hearts which were perfused, with rhythmic pressure, through the coronary arteries. This method is sufficiently accurate for comparative purposes; absolute measurements are not easy.

Langendorff (63) noticed that the perfusion pressure varied rhythmically during the contractions of the isolated hearts. This he assumed to be due to changes in resistance of the coronary blood vessels on account of compression by the contracting muscle. He studied these changes of pressure by connecting a side-branch of the perfusion cannula to a Hürthle's manometer and simultaneously recording the heart beat.

e. Experiments on heart-lung preparations and on the whole animal. Many physiological and pharmacological problems can be investigated on the isolated hearts, but the heart in this preparation is not under normal conditions, particularly since it does not perform any external work. We owe to Morawitz (85) the introduction of a method for measurement of the coronary blood flow in the whole animal. He inserts a special tampoon cannula through the right auricle into the coronary sinus, thus draining the major part of the coronary blood. This method has been extensively used in conjunction with heart-lung preparations; indeed it is in this preparation that Morawitz's method

proved to be the most useful especially since it was found by Starling and others that the outflow of blood from the coronary sinus stands under varying conditions in a more or less constant relation to the total coronary flow. De Barenne (27), (28) succeeded in measuring the total coronary outflow by means of a modification of Starling's heart-lung preparation[1] (58). By perfusing the preparation through the peripheral end of the pulmonary artery de Barenne freed the right side of the heart from all blood except that emerging from the coronary vessels. In this modification it is only the left ventricle which performs work, nevertheless the results obtained were valuable as corroborating the observations made on the ordinary heart-lung preparation.

Henriques (12, 46) attempted to determine the coronary flow by measuring the difference between the blood flow in the pulmonary artery and in the aorta. His experiments were however performed either on the isolated heart or on the whole animal after tying of all branches of the thoracic aorta. Recently a similar method was used by Anrep and Balatao (2) on the heart-lung preparation in which the pulmonary blood flow was measured by a suitable Stromuhr. This method allows a measurement of the whole coronary blood flow, it is therefore comparable with de Barenne's method but has the advantage that both ventricles perform mechanical work. The essential condition for a satisfactory use of the method consists in maintaining the inflow of blood into the vena cava constant, which is easy in the heart lung preparation but presents great difficulty in the whole animal. The study of the innervation of coronary blood vessels had been attempted on isolated hearts a procedure which is unreliable, though in the hands of Maas it gave certain valuable results (80). A considerable number of experiments were performed for the same purpose on the whole animal by Morawitz and Zahn (86), who drained the coronary sinus; Wiggers (126) and later F. Müller (88) collected the blood emerging from a cut coronary vein. In both cases it is difficult to control the hemodynamic conditions, the results obtained on the whole animal can therefore be regarded as only indicative and not conclusive.

Recently for the study of the coronary innervation Anrep and Segall

[1] The heart-lung preparation was first introduced by Martin (1883), later it was used in Ludwig laboratory by Stolnicov (1886) and Pavlov (1887), then again by H. E. Hering (1898), it became however a reliable standard preparation only after the important improvements which were introduced by Starling (1912).

(5, 6) introduced the innervated heart-lung preparation which differs from the denervated preparation in that the cerebral circulation of the animal continues to be maintained. In this preparation the heart and the brain are supplied from two separate sources and can be controlled independently. The heart retains all connection with the central nervous system, so that the preparation can be used for the study of the central and reflex regulation of the coronary circulation.

THE PROPERTIES OF THE ISOLATED CORONARY ARTERIES. The isolated coronary blood vessels have been studied by Langendorff (64), Campbell (18), Cow (23), Barbour (8), de Bonis and Susanna (29), Park (94), Pal (92), Eppinger and Hess (35), Ishihara (53), Cruickshank and Subba Rau (24). Most of these workers used coronary arterial rings for pharmacological studies. There are, however, a few physiological observations showing that the coronary arteries behave in several respects differently from at least some of the systemic arteries.

a. The reaction of isolated coronary arteries to changes in temperature. Cruickshank and Subba Rau compared the reactions of coronary and systemic arterial rings to changes in temperature; they found that while the systemic arteries (carotid, mesenteric and renal) when removed from the body are always in a state of contraction, the coronary arteries are relaxed. It was observed by MacWilliam (76) and later by Meyer (82) and by Cow (23), that on gradually increasing the temperature, the systemic arteries first slightly contract (at about 25°–30°C.) and then with further rise in temperature up to 45°C. they progressively relax. These observations were corroborated by the workers who studied isolated perfused organs. Lewashaw (67), Piotrovski (96), Pissemski (97), Lui (73), Berti (11) and others agree that a gradual rise in temperature of the circulating fluid brings about a very definite vasodilatation. The coronary artery reacts within the physiological range of temperature quite differently from about 22°C. and up to about 38°C. it gives a steady contraction which in fresh arteries is so intense that the diameter of the coronary arterial ring at 38°C. may be reduced to half the diameter at 22°C. From 38°C. up to 45°C. the coronary artery slightly relaxes.

On the whole these experiments bring out one main point of difference between the coronary and systemic arteries, that within the physiological range of rise in temperature, the systemic artery relaxes, while the coronary artery contracts. In other words when excised from the body the coronary artery is completely relaxed, while the systemic arteries are nearly fully contracted. These observations were

made on freshly isolated arteries of the dog, ox, and man (3 to 12 hours after death), as well as on arteries up to 48 hours old. In the latter case the difference between the systemic and coronary arteries was still quite definite. The results of these experiments should not be regarded as showing a fundamental difference between the coronary and systemic arteries in general, since it is possible that there are some other arteries in the body which react to temperature similarly to the coronary arteries. The observations are however important since they provide an interpretation of some other experiments upon the effect of temperature on the coronary circulation in the beating heart. All the changes due to temperature are reversible below about 45°C., while above this temperature they are irreversible; approximately at this temperature also drugs cease to have any effect. The following table has been compiled from the observations of Cruickshank and Subba Rau.

Changes in tone of the isolated arterial rings related to the tone of the artery at 22°C

TEMPERATURE	SYSTEMIC ARTERY		CORONARY ARTERY	
	Base line		Base line	
	Below	Above	Below	Above
22°–30°C.		+		+
30°–38°C.	−			+
38°–44°C.	−			−
44°–50°C.	+	or +		+
50°–60°C.	−			−
About 61°C.	+			+

This table summarises the general behaviour of the isolated arterial rings. However, as was already pointed out by MacWilliam, any general rule can be only approximate since isolated arteries under apparently identical conditions show wide variations in their reaction to temperature.

b. Effect of H-ion concentration. (24), (55), (74). Coronary arterial rings which are kept at a constant temperature (37.5°C.) show a definite contraction with increase in pH from 7 to 7.5 and a still further contraction with increase in the pH up to 9.0. Between pH 7.0 and 6.0 the tone of the arterial rings does not change appreciably, but below pH 6.0 it again increases. Above pH 7.0 and below pH 6.0 the minimal difference in the H-ion concentration to which an artery reacted was of the order of 0.2.

c. Effect of oxygen. (24), (88). At room temperature the coronary artery retains its relaxed state in oxygen free Ringer (pH-7.4) while the systemic artery under these conditions remains contracted for many hours. Warming the coronary artery to 37°C. in the absence of oxygen results in only a small contraction, while the systemic dilates very much more than in the presence of oxygen. Administration of oxygen produces in both cases a contraction, but the coronary artery contracts above the original base line while the systemic usually does not reach its base. The main importance of the observations upon the effect of H-ion concentration and of oxygen lies in their corroborating other observations performed on the beating heart. Very often the coronary rings exhibit rhythmic contractions similar to those described for systemic arteries (Müller, V. Frey, Meyer, Pal). In the coronary artery these contractions usually appear at about 39° to 40°C. and disappear again at about 44° to 45°C.; they occur only in the presence of oxygen. Presence of adrenaline increases rhythmic contractions which in this case may start at a considerably lower temperature. No comparative studies of the elasticity of the coronary and systemic arteries have been made.

Experiments performed by Subba Rau on arrested strips of rabbits' and cats' hearts corroborate the results observed on coronary arterial rings—the reaction to temperature changes, to oxygen, and changes in the H-ion concentration being of the same character. The strips were arrested by excess of potassium or lack of calcium, or they were taken from hearts which had stopped spontaneously after a few hours of coronary perfusion. The results were in all cases comparable.

CHANGES IN THE CORONARY CIRCULATION IN RELATION TO EVENTS IN A SINGLE CARDIAC CYCLE. During the past two centuries physiologists interested in the coronary circulation directed almost entirely their attention to the question whether the flaps of the semi-lunar valves in the aorta interfere in any way with the coronary circulation and whether the coronary blood flow is greater in systole or diastole. Scaramucci (116) advanced the theory that the coronary vessels are emptied by the contraction of the heart and fill during its relaxation. Stroem (120 a), Thebesius (123), Vieussens (124) and later Morgagni (87) expressed the view that during systole the coronary arteries are prevented from filling with blood by the closure of their orifices by the aortic valves. Lancisi (65) however doubted the correctness of this view, since he found on examining the position of the valves, that in many cases their edges were below the openings of the coronary arteries. Haller (41, 42, 43) and Hamberger (44), observing that the

pulsation of the coronary artery is synchronous with that of any other artery of the body, concluded that the aortic valves do not close over the coronary orifices and that the coronary flow must therefore be greater in systole. This point of view was supported by most of the anatomists of the nineteenth century (21), (25), (56), (66), (81). Stroem's theory was again revived, however, by Brücke (16), Marshall-Hall, Wittich (129) and received some support from Ludwig. Physiologists, however, on the whole refuted this view on grounds of experimental evidence. Hyrtle (50, 51, 52), Endemann (34), Budge (17), Perls (95), Rudinger (108) and Ceradini (19, 20) observed the changes of the blood pressure or of the blood flow in the coronary artery. They connected the central end of the coronary artery to a mercury manometer or they observed the spurting of blood from an incision in the coronary artery; in both cases they found that the rise in the coronary arterial pressure and the augmentation in the blood flow were synchronous with the cardiac systole. The experiments were repeated on excised heart in which it was possible to show that in systole the aortic valves do not close up over the coronary orifices. Rebatel (106), working under the direction of Chauveau, was the first to study the coronary blood flow with a more precise method. He inserted into the right coronary artery of the horse a T-piece which was connected to a haemodromograph; simultaneously he measured the blood pressure either in the aortic or in the coronary artery. The coronary blood flow so recorded showed two waves—one coincident with the beginning of the upstroke in the aortic pressure, and the second occurring late in diastole of the heart. Rebatel regarded the first wave as due to the momentum imparted to the fluid by the contraction of the ventricle, while he suggested that the second wave was due to an inrush of blood following a sudden diminution of the peripheral resistance in the intramuscular coronary branches. Klug (57) made an attempt to determine the degree of filling of the coronary system during systole and diastole by histological methods. He placed ligatures round the auriculo-ventricular junction in hearts of rabbits; in some experiments the ligature had to be tightened during systole, in others during diastole. This would seem to us to be an exceedingly difficult if not an almost impossible task to perform. On histological examination of such hearts Klug claimed that during systole the coronary blood vessels were nearly empty, while during diastole they were well filled with blood. Martin and Sedgwick (78) recorded simultaneously curves of the blood pressure in the carotid artery and in a branch of the left

coronary artery; their tracings were, however, taken with a mercury manometer. They observed complete synchronism of the pulse waves in the two arteries. Porter (99) repeated these experiments using a Hürthle membrane manometer; and found that "the most careful scrutiny of the two curves taken during the ordinary contractions or during slowing of the heart beat by vagus excitation failed to reveal any noteworthy difference, except that the pulse wave reaches the coronary artery sooner than the carotid, depending of course on the nearness of the former vessel to the heart." Extending his observations to the study of isolated beating strips of the ventricle of dog and cat, Porter found that at each beat some blood was forced out of the severed vessels in the margins of the strip. He repeated these experiments on the living animal, and observed exactly the same when the outflow of blood from an incised coronary vein was carefully watched. This effect of squeezing of the vessels by the contracting muscles was also observed in the case of incision of a coronary artery: at each contraction blood was expressed from the distal end. Porter thus arrived in 1898 at the same conclusion as did Scaramucci in 1689.

Langendorff (63) working on isolated hearts of cats studied both venous outflow and arterial inflow. With regard to the outflow he supports Porter's observations and attributes the increase in the outflow during systole to the mechanical effect of the cardiac contraction. With slowly beating hearts he observed the increase in the outflow to begin at the end of diastole. Langendorff was the first to note a certain effect of the auricular contraction upon the coronary blood flow. Reid (107), Gruber (40), Vintschgau (125), had at that time demonstrated a close association of the musculature of the coronary sinus with that of the auricle. Langendorff found that during auricular systole the outflow from the sinus is completely arrested on account of its closure by the surrounding muscles. Langendorff was the first to study the inflow of blood into the coronary arteries; he observed that the perfusion pressure varied with each cardiac contraction, and this he explained as due to the difference in the resistance to the inflow in the case of the contracted and relaxed ventricle. By simultaneously recording these changes in pressure and the contractions of the heart he demonstrated that the ventricular contraction favours at its beginning the inflow of blood into the coronary arteries. Then, according to the strength of systole, the resistance increases and the inflow diminishes. During diastole, owing to the reduction in the resistance, the blood flow

again increases. These relations are presented by Langendorff in the following table:

TIME IN SECONDS	HEART	CORONARY BLOOD FLOW
$\frac{1}{6}$	Isometric period	The inflow of blood into the vessels is facilitated. The outflow from the coronary sinus is possible
$\frac{2}{6}$	Ejection phase	Inflow impeded; outflow increased
$\frac{1}{6}$	Diastole	Inflow increased; no outflow on account of the filling of the blood vessels
$\frac{2}{6}$	Auricular systole	Inflow increased; no outflow owing to the closure of the coronary sinus

Langendorff's conclusions could not be applied to the heart in the whole animal, especially, since it is possible that in the latter the systolic increase in the aortic pressure could antagonise the increased resistance afforded by the contracting heart. Furthermore there can be hardly any doubt that his records were vitiated by the great inertia or the moving column of fluid, by the neglect of the probable leak through the aortic valves and by the actual movement of the heart at the end of the cannula. For over twenty-five years the subject rested as left by Langendorff until lately the study was again revived in this laboratory. Anrep, Downing, Cruickshank and Subba Rau (3) made an attempt to measure the exact time relations between the changes of the coronary outflow and inflow on the one hand and the different phases of the cardiac cycle on the other. The method used was that of the hot-wire in the form of a sensitive anemometer (3), (4). After performing different preliminary tests, a method was devised by means of which the changes in the coronary outflow and inflow could be determined within a time interval of about 0.01 second. The registration of the coronary blood flow was taken in the different experiments simultaneously with the optical records of pressure changes in the left and right intraventricular cavities, in the auricles, in the aorta, and with records of the electro-cardiogram. The outflow and inflow were studied under different conditions of the heart beat, such as premature contraction, heart block, ectopic rhythms, auricular and ventricular fibrillation. The experiments were performed upon the heart-lung preparation.

The coronary outflow. The blood issuing from the coronary sinus was drained by a Morawitz cannula and allowed to fill a bottle which was stoppered by a hot wire container. On examining the records of the coronary outflow it can be noticed that three positive waves occur

during a single cardiac cycle; the first positive wave is pre-systolic, while the second and third waves occur within the period of contraction of the heart. In many cases the first two positive waves are separated by a phase of considerable diminution in the outflow which the authors call the negative wave. There is, of course, no back flow into the coronary sinus during this phase. The relations between the magnitude of these three waves may vary, but usually the second wave is the smallest, the next in size being the pre-systolic wave while the largest is the third. The negative wave is generally observed only when the pre-systolic wave is well-marked. The first or pre-systolic wave of the coronary outflow is determined by the auricular contraction. It disappears in auricular fibrillation; in 2:1 or 3:1 heart block it follows the rhythm of the auricle; on simultaneous registration of the auricular pressure it is found to occur in the second half of the auricular systole, and on correlating the outflow curve with the electro-cardiogram it is found to follow the P wave of the latter. If the ventricle is rythmically stimulated by single induction shocks so that the ventricular contraction precedes that of the auricle the pre-systolic wave becomes superimposed upon the third wave of the coronary outflow, or in many cases becomes nearly indistinguishable. The magnitude of the auricular complex of the coronary outflow depends upon the strength of contraction of the auricles, and upon filling of the coronary sinus. In experiments in which the coronary sinus was allowed to contain only very little blood the auricular complex becomes much less conspicuous. The authors do not commit themselves to an interpretation of the mechanism by which the auricular contraction affects the coronary outflow but it seems highly probable that the effect is due to compression of the coronary sinus by the auricular muscle which stands in close connection with the whole length of the sinus. It is difficult to determine whether the first positive wave occurs under normal conditions when no cannula is introduced into the sinus and when the sinus outflow is subjected during the auricular systole to an increased pressure. However, it is more probable that the first wave does occur also under these conditions, especially since it takes place late in the auricular systole when the intra-auricular pressure is already diminished.

The wave of diminished outflow (the negative wave) has been regarded as an after-effect of the first positive wave; it is probably determined by the degree of emptying of the coronary sinus during the auricular systole. The negative wave begins within the period of the auricular contraction and continues up to the onset of the isometric

contraction of the ventricle. The beginning of the wave is not very definite, its end is, however, very sharp. In relation to the electrocardiogram the negative wave of the outflow ends at the peak of the deflection S.

The beginning of the small second positive wave coincides with the contraction of the ventricles. The duration of this wave depends on the magnitude of the coronary blood flow. The larger the inflow, the more does the length of the second positive wave approximate the duration of the isometric contraction of the ventricles.

The third and the largest positive wave begins abruptly, with large coronary flows its beginning is almost coincident with the opening of the aortic valves. The wave reaches its peak still within the period of maximum ejection and then abruptly falls off.

On the whole these observations support the conclusions reached by Porter and Langendorff in so far as they show that the maximal coronary outflow occurs during the period of systole. They add some new details and differ from Langendorff's point of view as to the effect of auricular contraction upon the coronary outflow. Langendorff on simple inspection thought that the outflow is completely arrested during the period of auricular systole, whereas hot wire records show that the auricular complex consists of a diphasic variation, first an increase and then a decrease in outflow. It must be mentioned, however, that in slowly beating hearts Langendorff observed some augmentation of the outflow towards the end of diastole, and this might have been in reality caused by the auricular contraction.

The authors advance a tentative explanation of the mechanism determining the auricular complex of the coronary outflow. As regards the large third positive wave the mechanism cannot be determined without a simultaneous registration of the inflow of blood into the coronary arteries.

The coronary inflow. For the purpose of studying the inflow the aorta was connected in the heart-lung preparation with a reservoir containing blood. This reservoir was placed at a suitable height, and a cannula was inserted into the central end of the pulmonary artery and connected to the heart-lung apparatus, the blood flow being thus diverted from entry into the left heart. In such preparations it is only the right side which performs external work. In order to have both ventricles working the left ventricle should be connected to a second artificial resistance by a cannula placed directly through its walls. In the latter modification both ventricles work in as nearly normal conditions as possible.

If the constant pressure in the aorta is kept above the systolic pressure in the ventricle the valves remain permanently closed and in contrast to the case of isolated heart, leaks are negligible. The coronary inflow can thus be studied by measuring the rate at which the reservoir empties itself of blood.

As a result of such experiment it became possible to show that none of the typical changes in the coronary outflow depend upon the variations in the aortic pressure occurring during a cardiac cycle. The three positive waves were equally well marked in the case of constant aortic pressure as under normal conditions. It is obvious, therefore, that the large third wave is not caused by the systolic rise in the aortic pressure but by changes occurring in the heart muscle itself. During the third wave of the outflow, the coronary inflow was found to be considerably diminished and in the case of small coronary flows even arrested.

During the period of isometric contraction the coronary inflow is not changed; the earlier the diminution of the inflow begins during the ejection phase, the larger is the coronary blood flow. The minimal inflow occurs at the point of maximal contraction of the heart, i.e., late in the ejection phase and during the first part of the period of isometric relaxation. The augmentation of the inflow which at first is rapid, becomes soon slower and finally reaches a uniform magnitude which is maintained until the next ventricular systole takes place. No auricular complex could be observed in the inflow curve except in a few experiments in which a small and indefinite fluctuation was recorded simultaneously with the auricular systole.

During the ejection phase the blood pressure developed within the heart, and therefore the force compressing the intra-muscular blood vessels must be (during this phase) greater than the pressure in the aorta. It is therefore to be expected that the coronary blood flow through the left ventricle must be completely arrested during that period. However, on comparing the haemodynamic relations in the right and left ventricle it should be clear that the compressing effect of the right ventricle upon the coronary blood vessels must be at any time much smaller than that of the left ventricle. Moreover the coronary blood vessels which lie directly under the visceral layer of the pericardium must be subjected to a smaller compression than the deeper vessels. It should be expected, therefore, that the blood flow through the coronary blood vessels of the right heart should be affected, if at all, by the systole to a much smaller extent; while in the left ventricle the flow should be zero during maximal contraction of the heart. No

method however is available for the determination of the coronary blood flow through the right and left ventricles. These experiments in their main points support the observations of Langendorff. They show also that the coronary inflow is not determined by the mean aortic pressure, but by what may be termed the mean post dicrotic pressure. Further corroboration of this statement is found in the yet unpublished experiments performed with the same method upon the effect of aortic insufficiency.

I am informed that Dr. F. M. Smith of Iowa obtained similar results working on the whole animal. The aortic pressure was altered in his experiments by means of adjustable clamps on the descending aorta. He found that changes in the systolic pressure had no effect upon the outflow per minute from the coronary sinus; on the other hand, lowering of the diastolic pressure produced by means of arterio-venous anastomosis or by tearing the cusps of the aortic valves produced a very considerable diminution in the coronary flow, even in cases when the systolic pressure was artificially increased. G. Bourne (14) on purely theoretical grounds arrived at the same conclusions.

THE EFFECT OF MECHANICAL FACTORS OF CIRCULATION AND OF CHANGES IN THE HEART BEAT UPON THE CORONARY BLOOD FLOW. The study of the different changes in the coronary outflow and inflow occurring within a single cardiac cycle cannot throw much light upon the factors which determine the total volume of coronary circulation. The effect of different cardio-vascular factors upon the coronary blood-flow has been mainly studied on the heart-lung preparation in which the mechanical conditions of circulation, as well as the composition of the blood and the heart-rate, are under control. The minute blood flow was usually determined by draining the coronary sinus with a Morawitz cannula. Morawitz (85) was of the opinion that by his method the whole of the coronary blood was conducted outside the heart, so that in determining the outflow from the cannula, one was determining the total blood supply to the cardiac muscle. It must be remembered, however, that the large posterior cardiac vein opens into the coronary sinus close to its orifice, so that the blood emerging from this vein generally misses the cannula. In other cases, the vein may be partially obstructed, and it is doubtful on which side of the obstruction the blood will flow (71). Besides these large veins there are the veins of Thebesius, which open directly into the auricles and both ventricles, and which, as Pratt (104) has shown, can serve as efficient channels of nutrition in the isolated heart. The outflow from the coronary sinus

represents only a fraction of the total flow. Starling and Lovatt-Evans (72) find that the outflow from the coronary sinus is always greater than half, and generally less than two-thirds, of the total flow. On examining a great number of hearts they found that on an average three-fifths of the total coronary blood-flow passed through the coronary cannula, whilst about two-fifths was missed by this method. The ratio between these fractions varies slightly from heart to heart; it is important to note, however, that in any single heart it remains constant under widely varying conditions. For instance, with change in the total coronary flow from 32 to 177 cc. per minute, this ratio varied only between 0.51 and 0.67, and the direction of this variation was not directly related to changes in the total coronary circulation. These results were subsequently confirmed by experiments in which the coronary flow was determined by means of a stromuhr or by the hot-wire method. Nakagawa (90) also reports that with very large increases in the arterial blood pressure or in the case of maximal outputs of the heart, the ratio may change from about 0.7 to 0.5.

a. The influence of arterial pressure upon the coronary circulation. It is, a priori, evident that the aortic blood pressure must constitute the main factor in the control of the coronary circulation. Langendorff and Porter had already noticed that an increase in perfusion pressure greatly augments the coronary flow in the isolated heart. In the heart-lung preparation, Markwalder and Starling (79) found that the coronary flow was readily influenced by changes in the mean arterial pressure. While, for instance, at a pressure of 40 to 60 mm. Hg, the total coronary flow in an average sized dog's heart (50 to 60 grams) may be of the order of 15–20 cc. per minute, with a rise of pressure to 120–140 mm. the flow may increase to as much as 250 cc. per minute or more. With a gradual rise in arterial pressure the coronary flow at first increases slowly; then above a pressure of about 100 to 120 mm. the increase is more and more rapid. Morawitz and Zahn (86) and Bond (13) found a similar dependence of coronary flow upon the mean arterial pressure in the whole animal. De Barenne (28), (29) using his modification of the heart-lung preparation, and later Anrep and Bulatao (2), using the stromuhr method—both therefore measuring the total coronary blow—confirmed these results.

Many attempts have been made to arrive at a figure which would adequately represent the volume blood-flow per gram of heart per hour under normal conditions. Such a calculation is rendered difficult since, as will be seen later, there are many other factors which influence

the coronary circulation in addition to the arterial pressure. It seems, however, justifiable to take a flow of 40 to 60 cc. per gram per hour as representing, on an average, the magnitude of the coronary circulation in the heart-lung preparation at an early stage of the experiment and at a blood pressure of 100–110 mm. This value, however, may vary considerably in both directions in different experiments: it cannot be applied to heart-lung preparations later in experiments, nor to the whole animal with intact cardiac nerves.

Hot-wire records of the coronary outflow show that its typical variations, taking place within a single cardiac cycle, are not appreciably changed by a rise in the arterial pressure. The three positive waves become somewhat more accentuated, while the duration of the second wave is shortened approximating to the isometric period of contraction. The whole curve is, however, considerably shifted upwards, showing that a rise in the arterial pressure affects the coronary outflow equally in diastole and in systole.

b. The influence of the cardiac output (venous inflow) upon the coronary circulation. In contrast to the predominant influence of arterial blood pressure, variations in the cardiac output have, in the denervated heart, an almost negligible effect upon the coronary flow; and even those changes which are observed (about 2 to 4 cc. per minute) may be accounted for by the difficulty of maintaining the mean diastolic blood pressure constant with different cardiac output. In the heart-lung preparation the outflow from the sinus, as well as the total coronary flow, remained practically constant with variations in output ranging from about 100 to 900 cc. per minute in Nakagawa's experiments (89), and to over 1200 cc. per minute in Anrep and Segall's experiments (6). These observations were confirmed by all those workers who used the heart-lung preparation. Hot-wire records show that the configuration, as well as the level of the curves of outflow and inflow, remain unchanged with wide variations in the cardiac output. The auricular wave becomes somewhat more evident, the second and the third waves exhibit no change either in their duration or in their height. It is obvious that in such experiments with the hot-wire method, in order to obtain identical curves of coronary outflow with different cardiac outputs, the rate of the heart and the temperature of the blood must be constant.

The independence of the coronary circulation in the denervated heart of changes in output raises several interesting points. According to Porter (99) and Wiggers (126), an important part in the circulation through the coronary vessels is played by the massaging action of the

heart-muscle during its contraction. Porter, on the basis of his experiments, as well as on the results of the experiments by Magrath and Kennedy (77) concludes that an augmentation in the force of the heart beat increases the blood flow through the coronary system. It is obvious that an increase in the cardiac output, as shown by Frank (36, 37), Starling, Piper and Patterson (118), H. Straub (120) and Wiggers (127), is primarily associated with increased force of cardiac contraction. The observations of Porter and his followers, and of those workers who use the heart-lung preparation, are contradictory. The reason for this discrepancy is not clear, but it may be explained by the fact that Porter and his co-workers performed their experiments only on isolated perfused hearts, without controlling the usual leak through the aortic valves. On examining the only curve that is given by Porter, it can be seen that when he produced an increase in the left intra-ventricular pressure, maintaining at the same time the coronary perfusion pressure constant, though the heart beat itself increased in strength, the coronary flow remained unchanged, and it was only when the heart rate abruptly slowed from about 240 to 120 beats per minute that the coronary circulation definitely increased [Porter (99) p. 158, fig. 4]. According to Porter "the increased intra-cardiac pressure caused the ventricle to beat with greater force, and therefore the volume of the coronary circulation became greater,—and this in spite of a diminished frequency of contraction." In the light of newer observations it is more probable that the coronary circulation increased because of and not in spite of, the diminution in heart-rate. Magrath and Kennedy's experiments are often wrongly cited as showing dependence of coronary flow on cardiac contraction. In their experiments, however, the increase in the strength of contraction was produced not by an increase in the diastolic length of fibres, but by a rise in the coronary perfusion pressure, which, of itself, would be sufficient to produce the effect. Though the heart beat as a result of a better perfusion did increase in strength, it is sufficient to consult their tracings to see that the increase in coronary flow followed immediately on the increase in perfusion pressure and not on the augmentation of the heart-beat, which occurred at a considerably later stage. Hyde (49) demonstrated that a rise in the intra-ventricular pressure is accompanied by a diminution in the coronary flow, even if the increase be so small as 5 mm. Hg, a change sufficient to diminish the coronary flow by approximately 20 per cent in spite of the concurrent increase in the force of the heart beat. The observations of Hyde were confirmed in this laboratory in experiments performed with her tech-

nique. In the author's opinion there is at present no evidence in favour of the view that physiological increase in the strength of the cardiac contraction is attended by a corresponding change in the coronary flow. So far as the denervated heart-lung preparation is concerned, it can be considered as definitely proved that changes in the strength of the heart beat as produced by changes in the stroke volume have no effect on the coronary circulation.

The observations of Lovatt-Evans and Matzuoka (71) recently repeated by Starling and Visscher (119), show that an increase in the work performed by the heart is accompanied by increased oxygen consumption, the magnitude of which is determined, within physiological limits, by the diastolic volume, the mechanical efficiency increasing with increase in work. Since increase in work produced by a rise in arterial pressure is accompanied by an augmentation in coronary blood-flow, while the increase in work resulting from an augmented output is not, it follows that in the first case the increase in oxygen consumption may be effected with a much smaller rise, if any, in the coefficient of oxygen utilisation than in the second case, in which the entire rise of oxygen consumption can be met only by an increase in the coefficient of oxygen utilisation. It seems therefore that the denervated heart, in contrast to the innervated heart, as will be shown later, has a one-sided mechanism for adapting its blood-flow to its expenditure of energy.

These observations, in accordance with the experiments of Hilton and Eicholtz (47), make necessary a thorough re-investigation of the problematic influence of physiologically produced "metabolites" upon the coronary circulation.

c. The influence of heart-rate upon the coronary circulation. Porter, working on the isolated heart, found a direct relation between coronary flow and alteration in heart rate, which occurred spontaneously or as a result of vagal stimulation. Subsequent workers changed the heart-rate by 1, variations in the temperature of the blood; 2, heating or cooling of the sino-auricular node; 3, rhythmic stimulation of the heart with single induction shocks. The effect of change of temperature of the circulatory fluid was studied by Nakagawa (89) in the heart-lung preparation, and by Sassa (109) on the isolated cat's heart. On the whole, their results are in good agreement in showing that the coronary circulation invariably decreases with increase of temperature and consequent acceleration of the heart. For instance, in Nakagawa's experiments, a rise in temperature from 31 to 38° accompanied by an increase in heart rate of 120 to 190 beats per minute, diminished the sinus output from

24 cc. to 17 cc. per minute. On diminishing the temperature below about 31° the coronary flow again diminished, which may probably result from a change in the mean diastolic pressure. In Sassa's experiments, with coronary perfusion at a constant pressure, the coronary blood-flow did not show such a diminution below 31°, but continued to increase progressively. It is obvious that these changes in the coronary circulation cannot be ascribed without reservation to changes in the heart-rate, but rather to a direct effect of temperature on the coronary vessels. This receives direct confirmation from the fact that isolated coronary arteries react to an increase in temperature by a strong contraction and from the experiments of the author, in which a diminution of coronary flow accompanied an increase in temperature in a heart-lung preparation with the heart-rate strictly controlled.

Changes in heart-rate produced by warming and cooling of the sino-auricular node have according to Miyake (83), (84) no effect upon the coronary blood-flow. According to Sassa, acceleration of the isolated heart so produced causes a small augmentation, and retardation a small diminution in the coronary flow. However, the fact that these alterations in the flow were not synchronous with the changes in heart-rate, but "lagged" by about 20 to 30 seconds, was not adequately explained.

"Driving" of the heart at rates varying from 60 to 200 per minute has no effect on the volume of the coronary flow in the heart-lung preparation (6). Hammouda and Kinosita (45) confirmed these results for the isolated rabbit's heart, and found, moreover, that an increase in heart-rate above a certain limit produces if anything a small diminution in flow. Drury and Smith (31) working on the coronary circulation of the tortoise did not observe any change with increased heart-rate. Hot-wire records give a similar result, they also afford a tentative explanation of the effect of heart-rate, in showing that the coronary inflow and outflow per beat is proportional to the heart-rate, until a certain limit is reached when the "minute-diastole" (the total time-period occupied by the diastole in a minute) becomes so short that the coronary blood vessels have no time to fill completely.

d. The effect of arrest of the heart, and of ventricular fibrillation upon the coronary circulation. According to the observations of Porter and Langendorff the coronary circulation is considerably smaller in the resting heart as compared with the beating heart. Sassa noted that in a cat's heart in which the periods of contractions were intermittent, a temporary increase in the coronary flow during diastolic arrest, and a diminution on the resumption of the beat.

Hammouda and Kinosita report a considerable increase in the coronary flow in isolated hearts which spontaneously arrested in diastole. However, the flow does not remain at its high value for more than 2 to 5 minutes; it begins gradually to diminish, and reaches a minimum in about 15 to 30 minutes. It can be noticed that simultaneously with this diminution the heart gradually and spontaneously enters into a state of contracture, which finally brings about a greater shortening of muscle-fibres than that during the strongest contractions of the beating heart. In the case of hearts arrested spontaneously in systole, the coronary inflow also shows an increase in the circulation in the case of diastolic arrest of the heart; in these cases the inflow is maintained at the level of the maximal inflow observed at the end of each diastole in the beating heart. It appears, therefore, that the coördinated contraction of the cardiac muscle does in no way increase the coronary minute-flow, either directly or indirectly, but, on the contrary, tends to diminish the flow during each systole; especially as the coronary blood vessels, as shown by Porter, being emptied during systole do not exercise any suction which would facilitate their filling during the subsequent diastole.

Ventricular fibrillation produces a similar effect to arresting the heart; the coronary flow increases when the heart fibrillates in diastole, as was first found by Langendorff (63), Mass (80) and H. Fredericq (38) on the isolated heart, and subsequently confirmed by Sassa on the cat, Hammouda and Kinosita on the rabbit, Hilton and Eicholtz (47) on the dog, and also by the hot-wire method (3). Fibrillation which is accompanied by tonic contracture of the muscle brings about a diminution in the coronary flow.

THE INFLUENCE OF CHEMICAL FACTORS UPON THE CORONARY CIRCULATION. The chief evidence for the importance of changes in the gaseous composition of the blood rests upon the results of the perfusion experiments of Barcroft and Dixon (10), and those of Morawitz and Zahn (86) on the whole animal, and Markwalder and Starling (79) on the heart-lung preparation.

a. The effect of changes of oxygen saturation of the blood upon the coronary circulation. H. Fredericq (38) working on the isolated heart states that deprivation of oxygen in the isolated heart results in diminution of the coronary flow, while re-administration of oxygen augments the flow. These observations could not be confirmed by subsequent workers. Hammouda and Kinosita found a very considerable acceleration of the coronary flow on diminution of the oxygen content of the blood. In the heart-lung preparation, Hilton and Eicholtz report that

diminution of the oxygen saturation of the blood produces an immediate rise in the coronary flow, which may increase four or five times. A diminution in oxygen saturation from 100 per cent to 80 per cent causes only a small change in the coronary flow: the main effect of deprivation of oxygen occurs only when the oxygen saturation falls below about 50 per cent. In these experiments, the carbon dioxide tension and the H-ion concentration of the blood were maintained constant. It is highly probable that the effect of oxygen lack is direct, and not on account of increased accumulation of products of metabolism. An increase in the concentration of "metabolites" is to be expected when the tissue runs into oxygen debt as a result of an inadequate utilisation of oxygen. In the experiments of Hilton and Eicholtz, however, the oxygen consumption during the periods of diminished oxygen saturation of the blood showed little change, the increased flow keeping pace with, and compensating for, the reduction in the oxygen saturation. The heart can take oxygen from the blood till it is completely de-oxygenated, as previously shown by Lovatt-Evans and Starling (72). The coronary dilatation consequently cannot result from accumulation of "metabolites," and moreover, the immediate and considerable increase in the rate of coronary blood-flow caused by decrease of oxygen tension in the blood cannot be imitated by addition of carbon dioxide and lactic acid, the latter even in quantities far in excess of those actually formed in sudden anoxaemia.

In ventricular fibrillation anoxaemia has exactly the same effect as in the beating heart showing that the augmentation of the coronary flow is due to direct effect of oxygen lack upon the coronary blood vessels and not to changes in the heart beat. Administration of cyanides has a remarkably large dilatory effect upon the coronary blood vessels the onset of which is very abrupt. The coronary dilatation occurs even with such doses of cyanides which have no effect upon the heart beat. The authors (47) ascribe the effect of cyanides to severe anoxaemia of the tissue.

b. The effect of changes in the reaction of the blood upon the coronary circulation. Barcroft and Dixon (10) showed that increased carbon dioxide tension in the perfusing fluid increases the coronary flow in the isolated heart. This was confirmed by H. Fredericq and by Markwalder and Starling who worked on the heart-lung preparation. A change in the CO_2 content in the inspired air from 3 per cent to 7 per cent, associated with an increase of hydrogen-ion concentration from pH 7.7 to pH 7.35 produced an increase in the coronary flow from about 40 to

60 cc. per minute. It is difficult to make an exact quantitative comparison between the effects of anoxaemia and acidaemia; it is, however, highly probable that in such a condition as asphyxia the main factor responsible for the extreme dilatation of the coronary arteries is the lack of oxygen and not the increased carbon dioxide content of the blood: an increase in carbon dioxide tension unaccompanied by lack of oxygen causes only a small increase in the coronary flow. The effects of carbon dioxide changes in H-ion concentration and of oxygen lack as observed on the heart-lung preparation are in accordance with the results obtained on isolated coronary arteries.

c. The influence of "metabolites" upon the coronary circulation. Interest in "metabolites" of a non-gaseous nature has recently been revived as a result of the work of Lewis and Grant (68), and of Dale (26). With respect to the coronary circulation Morawitz observed a progressive increase in flow in the course of the experiment. In the heart-lung preparation this gradual and spontaneous increase is of usual occurrence. It is at present, however, impossible to state definitely the cause of this coronary dilatation: it may result from the disappearance or accumulation of some substance from the blood, or from a progressive weakening of the cardiac contraction. In this connection an experiment performed by Hilton and Eicholtz may be mentioned. After establishing a heart-lung preparation, these workers removed a part of the blood from circulation, and kept it at body temperature for one hour. The coronary circulation meanwhile increased from about 35 to 45 cc. per minute. The whole of the circulating blood was then drawn off, and replaced by the blood previously removed. No change in the coronary blood-flow, however, was observed. This experiment speaks against any possible effect of accumulated "metabolites."

It is known that freshly defibrinated blood, as was used in the above experiments, has a vaso-constrictor effect upon all arteries, including those of the coronary system (24), (38), (124). This effect is diminished after the defibrinated blood has been in circulation for some short time. This explanation does not apparently hold in the experiment quoted above, as the addition of fresh blood did not cause diminution in coronary blood-flow. Neither can the progressive diminution be explained as being due to destruction of the minute amount of adrenaline present in the circulating blood since adrenaline, in the dog, produces an increase in flow, and not a diminution. On the other hand, histamine and pituitary extract, in small doses, cause a diminution in the coronary cir-

culation, but the part played by these substances in causing progressive dilatation of the coronary arteries has not as yet been investigated. It must be remembered that in the denervated heart-lung preparation, the heart dilates progressively over a period of some hours: the force of each contraction is constant, under conditions of equal output and pressure, though performed at a greater diastolic length of fibres. Since it is known that the coronary flow is greater in the relaxed heart, it is conceivable that the progressive augmentation in flow in the heart-lung is primarily due to this weakening of the heart-muscle. It is also possible that the effect of carbon dioxide, in addition to its direct dilator effect upon the coronary vessels favours the coronary flow by causing dilatation of the heart. Such an explanation is substantiated by an experiment of Nash (91), who showed that artificial acceleration by rhythmic stimulation of the heart, which has dilated under the influence of high concentration of carbon dioxide, produces a considerable decrease in the systolic and diastolic volumes of the heart, and, at the same time, a diminution in the coronary circulation. These results are in contrast to those given by experiments in which the heart is under the influence of low concentrations of carbon dioxide. Under such conditions artificial acceleration does not much reduce the mean volume, and does not diminish the coronary flow. These results may afford some explanation of the changes in coronary flow observed in spontaneously dilated hearts.

Histamine, the appearance of which in the circulation under certain conditions is highly probable (26), acts upon the coronary blood vessels of various animals in an extremely different manner. In doses of about 1:5,000,000 histamine contracts the isolated coronary rings of the ox and dog. The blood vessels of the quiescent isolated ventricular strips of the rabbit contract most vigorously even with smaller doses. The dilator effect of adrenaline can be most effectively demonstrated in strips of rabbits' hearts, the blood vessels of which are maintained in a tonic state by a small addition of histamine to the perfusion fluid. In the cats' hearts, however, Gunn (40a), Cruickshank and Subba Rau (24) found histamine to produce a very considerable dilatation of the coronary vessels. In the tortoise histamine also dilates the coronary blood vessels as shown by Sumbal (122). The effect of different amines has been also studied by Lihacheva (69), (70) who found that in the rabbit histamine, phenylethylamine, tyramine, tryptamine, as well as choline and neuvine all cause a variable degree of contraction which is strongest with histamine, weaker with phenylethylamine and very evanescent or even

absent with the last two. The changes in the heart beat produced by these drugs bears no relation to their effect on coronary blood vessels. The effect of purine bodies was studied by Kravkov and by Iwai and

FACTORS ?	CHANGES IN THE MEAN HEART VOLUME	CHANGES IN THE CORONARY BLOOD FLOW	REACTION OF ISOLATED ARTERIES
CO_2 (79)	Large dilatation	Small increase	Dilatation
O_2 (47)	First no change then dilatation	Very large increase	Dilatation
Cyanide (47)	First no change then dilatation	Very large increase	Dilatation
Adrenaline (79)	Decrease	Increase	Dilatation
Histamine (45) (small doses) (48)	No change or indefinite change	Decrease in rabbit, increase in cat	Contraction (rabbit) Dilatation (cat)
Alcohol (121)	Dilatation	Decrease	Not determined
Increase in temperature (89)	Diastolic volume unchanged, increased or decreased	Decrease	Contraction
Increase in venous filling (6), (47), (89)	Increase	No change	
Decrease in venous filling (6), (47), (89)	Decrease	No change	
Acceleration by driving (6), (47)	Under normal conditions no change	No change	
Acceleration by driving of heart dilated by CO_2 (91)	Decrease (Diastolic volume diminished)	Decrease	
Arrest or fibrillation in diastole (45), (47), (109)	Increase	Increase	
Arrest or fibrillation in systole (45), (109)	Decrease	Decrease	
Strophanthin and digitalis (11a)	Decrease	Increase	Not determined
Caffeine (11a)	Transitory decrease	Lasting increase	Dilatation
Camphor (11a)	Indefinite	Increase	Not determined
Amyl nitrite (11a)	Increase	Increase	Dilatation
Insulin (11a)	Lasting decrease	Indefinite	Not determined

Sassa who found caffeine and theobromine to produce a considerable dilatation of the isolated coronary arteries (24) as well as of the coronary blood vessels in the arrested and beating heart of the cat, rabbit and dog (3), (54), (60), (110).

The coronary blood flow in relation to cardiac "tone" (mean heart volume). It was often suggested that the changes in tone of the cardiac muscle have an important effect upon the coronary circulation, an increase in tone (decrease in heart volume) leading to an augmentation of the coronary blood flow. Several observers advance the suggestion that the effect of different drugs upon the coronary blood flow may be due in many instances to alterations in tone of the cardiac muscle and not to a direct effect upon the blood vessels.

The table on preceding page, correlating the change in tone and change in the coronary blood flow under different conditions, shows that no definite relation between the action of drugs or changes in the heart beat on the one hand and coronary blood flow on the other can be traced. At any rate increase in the tone does not lead to increased coronary blood flow. In the author's opinion it is rather the dilatation of the heart which favours the coronary circulation; this effect may, however, in many cases be compensated by the direct effect of the drug upon the blood vessels.

The table shows that the action upon the coronary flow of at least the above mentioned agencies does not follow the change in the mean cardiac volume but follows the effect upon the isolated arteries. Changes in the condition of the heart muscle when producing dilatation of the heart are accompanied by increase in coronary flow and when producing a decrease in cardiac volume by a diminution in coronary flow.

THE NERVOUS CONTROL OF THE CORONARY BLOOD VESSELS. Microscopic observations show that, of all the arteries of the body, those of the coronary system have the richest innervation. Degeneration experiments performed by Woollard (130) prove that the coronary arteries, up to their finer ramifications, receive a double innervation. The larger arterial branches are innervated by vagal and sympathetic fibres to about an equal extent, while the innervation of the smallest branches is predominantly vagal in origin. The double innervation can be traced down to the arterioles while no nerve supply to the capillaries has been found.

Physiological experiments on the innervation of the coronary blood-vessels have been performed on the isolated heart, heart-lung preparation, and on the whole animal.

a. Vagal control. Panum is reported in Schmidt's "Jahrbuch für 1858" to have observed a contraction of the coronary arteries on vagal stimulation, while in 1869 Meyer observed a dilatation on

stimulation of the vagus (82a). Similar observations were made in 1891 by Martin (77a). Porter (103) claimed that the vagus nerve contains vaso-constrictor fibres. In later work, however, he became somewhat doubtful as to the correctness of this view: he was more inclined to explain the diminution of coronary flow on vagal stimulation as being due to the slowing of the heart. Mass (80) working on the isolated heart found that stimulation of the vagus causes, after a latent period of 10 to 20 seconds, a very considerable decrease in the coronary flow, even when the heart-rate had undergone no alteration. In the best of his experiments the coronary flow diminished by more than half. Wiggers (126) recording the coronary flow from a cut vein in the whole animal also found a considerable diminution in the coronary flow on stimulating the vagus nerve. His experiments are especially convincing since they were performed after the administration of atropine, and therefore under constant rate and strength of heart-beat. Nagakawa (89) stimulated the vagus in the heart-lung preparation for intervals so short as from 5 to 20 seconds, and though the heart slowed from about 120 to 170 to 23 to 65 beats per minute, the coronary flow remained unchanged. Anrep and Segall (6) working on the innervated heart-lung preparation, found the coronary circulation to differ in several important respects from that in the denervated preparation. In the first place with an equal arterial pressure, and a medium output the coronary circulation in the innervated heart is considerably smaller than in the denervated heart. The increased tone of the coronary blood vessels was found to be dependent on the integrity of the vagus nerves, and disappeared after their section. In the best experiments the coronary flow sometimes more than doubled. This increase does not depend upon the concurrent acceleration in the heart-beat, since an equal acceleration produced by rhythmic stimulation does not affect the coronary flow. Section of the vagi produced under conditions in which the heart-rate is not allowed to increase (artificial stimulation or small doses of atropine) nevertheless produces an augmentation of the coronary flow. Stimulation of the peripheral end of one vagus with rhythmic faradisation conspicuously diminishes the coronary flow after a latent period of about 20 seconds. If, however, the vagal stimulation is prolonged for more than two or three minutes the coronary flow begins again to increase, the heart rate remaining constant. The authors regard this as resulting from the presence of post-ganglionic sympathetic fibres in the vagus nerve, and find that if the vagus is stimulated high up in the neck, no such secondary augmentation occurs. The degree

of this diminution in the coronary flow is proportional to the strength of the stimulus and independent of the changes in the heart-rate: this occurs even if the heart-rate is artificially maintained constant during the period of vagal excitation. Injection of small doses of atropine, though abolishing the chronotropic effect of the vagus, does not paralyse the vaso-motor fibres. Larger doses of atropine, however, paralyse the cardio-inhibitory as well as the vaso-constrictor fibres. Increase in cerebral blood pressure, though possibly producing a slowing of the heart to the same or to a much greater degree, diminishes the coronary flow to a less extent than direct stimulation of the vagus.

The tone of the vaso-constrictor fibres of the vagus is subject to wide physiological alterations. In definite contrast to the denervated heart-lung preparation, the coronary flow in the innervated preparation is readily affected by alterations in the cardiac output, an increase in the output being accompanied by an augmentation of the coronary flow. This effect is independent of concurrent changes in the heart-rate (6) and is as conspicuous in experiments in which the heart-rate is strictly controlled. The augmentation in the coronary flow on increasing the cardiac output takes about 30 seconds to reach its maximum, at which it remains so long as the output is maintained constant. This effect irrevocably disappears after section of both vagi. The authors regard the effect as a reflex diminution in the vagal vasoconstrictor tone. The maximal increase in coronary flow following a large increase in output of the heart is of the same order as that produced by section of the vagi. It is therefore obvious that section of the vagi performed when the cardiac output is large, has a considerably smaller effect upon the coronary flow than when it is small. Extirpation of both stellate ganglion has no definite effect upon the coronary reflex. Along with the Bainbridge reflex (5), the coronary reflex is one of fundamental importance for the adaptation of the heart to large outputs. These two proprioceptive cardiac reflexes most probably play an important part in certain cardiovascular conditions as, for instance, in the case of muscular exercise in which the increase in the work of the heart is mainly associated with increased output, rather than with increased blood-pressure. The vaso-constrictor action of the vagus, though definite in the dog and very probable in the cat, has not yet been verified in other animals. Drury finds that in the tortoise the vagus supplies vaso-dilator fibres to the coronary blood-vessels.

b. The sympathetic control. Observations made on the action of the sympathetic nerves, and of adrenaline, on the coronary system, yielded

contradictory results. Schäfer (115) found that adrenaline had no effect on the coronary circulation. Mass (80) and Sassa (109) observed a vaso-dilatation on stimulation of the sympathetic in the isolated heart. Morawitz and Zahn (86) in the whole animal found a large increase in the coronary output on injection of adrenaline and on stimulation of the stellate ganglion. Dogiel and Archangelsky (30) observed a change in the size of the coronary vessels in the cat, dog and tortoise after bring ing the heart to a standstill by vagal stimulation and then stimulating the sympathetic. They state that under such conditions the size of the superficial coronary arteries was decreased. They published photographs of the heart before and after stimulation of the sympathetic, which, however, do not clearly show the results which these observers wish to illustrate.

All workers who have studied the effect of adrenaline on the beating heart, whether isolated or in the heart-lung preparation, report that this drug has a strong vaso-dilator action. Brodie and Cullis (15) found that small doses of adrenaline diminish the coronary flow; while larger doses cause diminution followed by prolonged augmentation. They regard this diminution as being due to a true vaso-constrictor effect of small doses of adrenaline. Hammouda and Kinosita, however, pointed out that this diminution coincides with the period of maximal augmentation of the cardiac contraction, which, by itself, would cause a decrease in the coronary flow. Moreover, in fibrillating hearts, adrenaline does not produce such a preliminary diminution in the flow, which, of course, should occur if resulting from vaso-constriction.

Wiggers (126) and Rabe (105), working on the arrested heart of the cat, found adrenaline to produce vaso-constriction only. Their results, however, are regarded by Hammouda and Kinosita as being due to compression of the blood-vessels by the muscle which, under the action of adrenaline, enters into vigorous contracture. If this contracture is prevented by arresting the heart with arecoline, adrenaline produces a dilatation similar to that observed in the beating heart. Kravkov, (6), Anichkov (1) and Savodsky (111), working on rabbits' hearts arrested with strophanthin found that adrenaline produced vaso-dilatation.

In the response to the action of adrenaline as to many other drugs, a species-difference probably exists. While the isolated coronary arteries of most animals react to adrenaline by dilatation, the larger human coronary arteries respond by constricting (8), the smaller arteries, however have been observed to dilate (24). Drury and his coworkers

found in the tortoise (31, 32, 122), and Barbour and Prince (9) in the monkey a pure vaso-constriction. Anichkov (1), Savodsky (111) and Sechenov (113) working on the resting human heart, found that the coronary blood-vessels of the new-born and of young children dilate on perfusion with adrenaline, while in most adult hearts, the coronary flow is diminished; no registration of the state of the cardiac muscle was, however, made. In one case, however (1), adrenaline produced a definite vaso-dilatation in a heart from a subject seventy years old. In the cases of arterial sclerosis and diphtheria adrenaline even in large doses ceases to have any effect on the coronary blood vessels. In the heart-lung preparation of the dog, adrenaline produces a very definite vaso-dilatatory effect, as also does stimulation of the stellate ganglia.

Stimulation of sensory nerves in the innervated heart-lung preparation, cerebral anemia and asphyxia of the brain are also accompanied by a very considerable coronary vaso-dilatation which is effectively prevented by extirpation of the stellate ganglia (6), (86). None of these effects are dependent on any concurrent changes in heart-rate.

The recent experiments upon the innervation of the coronary blood vessels demonstrate the importance of the vasomotor regulation of the cardiac blood-supply, which, under normal conditions, is subjected to the tone of the vagal constrictors, and can therefore be increased, not only by various chemical and mechanical factors, but also by a diminution of this constrictor tone as well as by stimulation of the sympathetic vaso-dilators. The vasomotor mechanism can, as shown by experiments on the innervated heart-lung preparation, be brought into action by influences acting directly on the central nervous system, as well as in a reflex manner.

The existence of a sympathetic vaso-dilator tone and its participation in the reflex adaptation of the cardiac blood vessels has not been demonstrated.

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A REVIEW OF RECENT ADVANCES IN THE STUDY OF BLOOD SUGAR AND DIABETES

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In a previous review of work on this subject, published in 1923,[1] the author dealt with the most important papers which had appeared during the previous four years. The scope of this problem is so enormous that it was only possible to touch lightly upon each branch and to summarise the main facts which had emerged from the mass of literature available.

Since 1923, this has increased to such a volume that a second review appears necessary. It is needless to add that, as before, it will only be possible to give the bare outlines of each branch: to cover the ground completely would need a text-book of some size and an idea may be gained of the interest which the subject has aroused by the fact that over 350 papers have appeared in different scientific journals in the past two years. However, an attempt will be made to gather up the thread into some sort of coherent story in the hope that it may be of some service to those who have neither the time nor the opportunity to refer to the original papers.

As in the previous review, the subject will be divided up by a number of sub-titles, the same general order being observed as before, but additional headings are now desirable. In the case of any reference to, or revision of a previously held view, made necessary in the light of recently discovered facts, this will be made clear, so that, for all practical purposes, the present review may be regarded as superseding that published two years ago. Attention may also be drawn to a certain amount of original work, carried out by the author and one of his colleagues, which is now referred to for the first time and which will appear in detail elsewhere. In order to simplify reference, a bibliography is given at the end of each section.

I. THE PRESENCE AND MANNER OF EXISTENCE OF THE SUGAR IN THE BLOOD. As is now well known, the normal concentration of sugar in

[1] Med. Journ. of South Africa, 1923, xix, 34–48, 77–84.

the blood of humans varies from about 0.08 per cent to 0.11 per cent, and averages 0.1 per cent. The actual amount varies in different individuals and in the same individual at different times. The fetal blood sugar exists at a lower concentration than that of the mother, but rises near the time of birth (1). In healthy infants, three to five hours after nursing, the amount varies from 0.085 per cent to 0.125 per cent, the mean being 0.107 per cent. During the first five days of life the average is 0.100 per cent (2). There is no relation between the sugar value and the term of pregnancy or the birth weight (3).

Brown (4) reports that, in fasting infants under two weeks old, the blood sugar averages 0.087 per cent and thereafter it gradually rises to the adult level. In ill-nourished infants, lower values are found.

In pigeons the normal limits are 0.149 to 0.258 per cent, the actual amount varying according to the species. The sugar content of the blood bears no relation to the size or weight of the bird, but the average is the same in the two sexes and does not vary with age (5).

The normal figures for the blood of domestic animals are well known and may be found in most text-books.

As regards the manner in which the blood sugar exists, the three theories which find support are *a*, that it all exists in simple solution as glucose; *b*, that part is in simple solution as glucose and the remainder is combined in some way with protein, and *c*, that part is in simple solution as glucose and the remainder is present as di- or polysaccharide. In connection with the first theory, a large amount of controversy has taken place with respect to the actual form in which the glucose is present. Hewitt and Pryde (6) consider that the two forms of glucose, α and β, which constitute the ordinary d-glucose are not present in the blood in a simply equilibrated aqueous solution, but v. Creveld (7) failed to confirm this hypothesis and Delaville and Richter-Quittner (8) conclude that, from the results of ultra-filtration experiments with the blood of normal and diabetic persons and animals, the sugar is present in true solution as the ordinary d-glucose.

The theory of Winter and Smith (9) has attained some importance, if only from the fact that confirmatory evidence is almost entirely lacking. These authors consider that the sugar of normal blood is a form known as γ-glucose and that it becomes converted, in diabetes, into the α and β forms. Little or no support appears to be forthcoming to this theory and Mozotowsky (10), Denis and Hume (11) and Thannhauser and Jenke (12) have all failed to confirm Winter and Smith's findings.

The latter authors have recently published further work (13) in which they state that, in diabetic blood, there appear to be complex sugars present. Their experiments show that, on mild hydrolysis, there is a considerable increase in optical rotation and, on preparation of the osazones, other crystalline forms, in addition to glucosazone, have been observed. In rabbit's blood, after injection of adrenalin, or thyroid, similar substances have been detected; if, however, both are administered together, it is stated that the type of blood sugar remains normal. It will thus be seen that these authors appear to have modified somewhat their original view.

Lundsgaard and Holboell (14) state that, in normal subjects, the sugar of the blood exists in a form with a lower rotatory power than ordinary glucose, but that, in diabetic blood, the optical activity shows no deviation from that of glucose.

A point which appears to have escaped notice is that, immediately the blood is shed, certain changes, about which very little is known, take place. Some of these changes are reflected in an apparent disappearance of the sugar from the blood or, at all events, a diminution of the reducing power of the blood. This will be dealt with later under the heading of Glycolysis and it is merely mentioned now to indicate that the possibility exists that such complicating factors may introduce serious obstacles to the problem under discussion. The changes mentioned take place, under certain circumstances, with great rapidity and, even if concordant results were obtained by different workers upon blood in vitro, it would still be difficult to speak with certainty upon the state of the blood in vivo. The point made here may possibly explain the failure on the part of workers in other laboratories to obtain results comparable to those of Winter and Smith.

Despite the amount of attention which has been paid to this problem, therefore, it remains at present unsolved and the nature of the blood sugar is still undetermined.

II. The distribution of sugar between corpuscles and plasma. Up to 1921 it was generally considered that the concentration of sugar in corpuscles and plasma was approximately the same. In the following two years this view changed to some extent as a result of further research and the balance of evidence favoured the view that, in normal blood, the corpuscles were almost, if not quite free from sugar, but that, in diabetes there might be a variable amount in the corpuscles. The experimental methods which have been adopted are open to considerable criticism. That from which the error is likely to be least em-

ploys the following technique. The blood is either centrifuged in a ligatured portion of a large vein, or received into paraffined tubes and centrifuged. The sugar content is determined in the whole blood and plasma, the percentage in the corpuscles being calculated by difference. Even in this case, however, some error is likely to arise, unless the separation of corpuscles from plasma is brought about with the utmost rapidity, since the tendency, immediately the blood is shed, is for an equalization of concentration to take place by diffusion.

Since 1923 very little work has been published on this question. Hansen (1) states that in 93 per cent of normal individuals (the actual number examined is not given) the sugar percentage in corpuscles and plasma was the same.

If sodium oxalate is used as anticoagulant, it is stated by Cristol and Danitch (2), that there is more sugar in corpuscles than in plasma, but if sodium fluoride is used and care taken to ensure hemolysis before the blood proteins are precipitated these authors found that equal volumes of plasma and corpuscles contain the same amount of sugar.

Hogler and Ueberrack (3) state that in the rabbit the proportion of sugar in the corpuscles is very small, compared with that in the plasma. By a method embodying the use of anticoagulants, it was found that human corpuscles contained 0.05 to 0.08 per cent of sugar.

In the absence of the original papers in the cases above referred to, it is impossible to criticise the results reported, but from the only experiments with which the writer is personally acquainted, it would appear that, if stringent precautions be taken to obviate diffusion as far as possible, the amount of sugar in the corpuscles is very small. An experiment, carried out by one of the author's colleagues,[2] full details of which will appear elsewhere, in which the "living test-tube" method of centrifuging the blood inside a section of vein was adopted, gave the following results: corpuscles, 0.06 per cent; plasma, 0.25 per cent. The high sugar content in this case is explained by ether anesthesia. Further work is being carried out on the same lines with human blood, the results of which at present indicate that the amount of sugar in the corpuscles is very small compared with that in the plasma.

It seems clear that, at the present moment, no definite opinion can be formed on this matter, but the balance of evidence weighs heavily against the possibility of there being any considerable quantity of sugar in the corpuscles.

[2] Mr. J. H. Dowds.

III. The concentration of sugar in arterial, venous and capillary blood. There appears to be little in the literature since 1923 dealing with this point. The conclusion reached then was that the concentration in arterial blood is usually slightly greater than in venous blood and that, ordinarily, no differences exist in the sugar content of venous and capillary blood. It is however desirable, in making a series of blood sugar estimations, that the blood in every case be taken from the same source, i.e., not on one occasion from a finger prick and on another by venepuncture. Only three papers have been published in the past two years on this subject. Lundsgaard and Holboell (1) and Frank, Nothmann and Wagner (2) confirm the previously held view that in normal subjects, venous blood shows a slight diminution from arterial in sugar content, but the former authors find that in diabetics no differences in this respect are observed.

From investigations made on normal dogs, Nitzescu and Cadariu (3) conclude that blood from the femoral or renal artery always contains more sugar that that from the corresponding vein.

It seems reasonable to conclude that the view outlined above on this branch of the subject is the correct one, since there is no contradictory evidence to oppose it.

IV. Physiological glycemia and hyperglycemia. A considerable variety of circumstances affects the concentration of blood sugar in normal persons and animals. The most frequent fluctuations occur as a result of the ingestion of food, but in normal individuals it is practically impossible to raise the sugar level above 0.17 per cent even by the administration of large amounts of glucose. As is well known, the height to which the blood sugar can rise before the kidneys begin to excrete it in the urine is spoken of as the renal threshold. This varies with different individuals and usually lies within the limits of 0.15 and 0.18 per cent but no definite figures can be laid down. Several other factors, which may be referred to as physiological, exercise an effect on the blood sugar concentration and the recent work on these will now be summarised.

Feinblatt (1) reports that, in 2000 routine examinations, 81 patients were found to have a blood sugar level of above 0.15 per cent, but only 42 per cent of these were diabetics. The same author emphasises the fact that it is unwise to place reliance on single examinations, in view of the transient hyperglycemia which is often caused by emotion. It is found also that, not only does the renal threshold vary in different individuals, but in the same individual at different times.

The alimentary hyperglycemia which occurs after meals depends on the nature of the food ingested. A level of 0.15 per cent is often found in complete health and the highest point of the sugar curve appears half an hour after the meal and frequently does not return to the normal in two hours (2). The effect of a normal meal has been studied by Brill (3) in a number of university students; this author found that, contrary to the usual rise, there was an actual fall in the sugar level 1½ hours after breakfast, amounting to an average of 4 mgm. After lunch, it was found that there was a rise. A meal of fat and protein only, in a normal person, is reported by Petren (4) to produce a rise in blood sugar.

The administration of glucose, as such, produces hyperglycemia of varying degree and other sugars may or may not react in this way. For example, lactose is stated by Holm (5) to have no influence in raising the blood sugar and the same author states that levulose has hardly any effect. The latter sugar, however, in dogs poisoned with phloridzin, causes a considerable rise (6). Glucose may be ingested in amounts insufficient to cause hyperglycemia; in this case Przylecki (7) states that the sugar is deposited solely in the liver, but amounts large enough to cause hyperglycemia induce the deposition of sugar in the muscles as well as the liver. This author has found the same results following the subcutaneous injection of glucose.

Albritton (8) injected glucose into unanesthetised dogs for a period of 2½ hours, in amounts equivalent to 0.7 gram per hour per kilo body weight. It was found that a rise in blood sugar took place in the first 20 to 30 minutes and was then maintained at a fairly constant level, about 0.05 per cent above the ordinary fasting level. It was found by Schatti (9) that the ingestion of 20 grams of glucose, levulose, sucrose, lactose or galactose in normal individuals produced hyperglycemia, the degree varying in the order stated. The same amount of starch, glycin or alanine had no effect in this connection. One hundred grams of starch produced the same amount of rise as 100 grams of glucose, but the effect was more delayed.

It will be noticed that there is some conflict in these results compared with those of Holm, as regards levulose and lactose.

Bennett and Dodds (10) found that 500 grams of glucose produced a rise in the blood sugar from 0.83 or 0.90 per cent to 0.114 or 0.125 per cent in half an hour. The curve then returned toward the normal. Hansen (11) states that the blood sugar after administration of glucose seldom rises in normal persons beyond 0.18 per cent and considers this

to be due to an acceleration of the removal of sugar from the blood which reaches its greatest capacity at about that level. This may be brought about by an increased supply of the pancreatic hormone. Under such circumstances a diminished metabolism is reported by Stricker and Bouckaert (12).

The reaction of the body to administration of glucose is made use of in the glucose tolerance test, which affords valuable indications of the state of carbohydrate metabolism. This will be referred to later in the section dealing with the treatment of diabetes. Operative manipulation has been found to cause fluctuations in the blood sugar level and in the earlier work on the subject it was difficult to determine what effects were due to the actual operative procedure, owing to a masking action, caused by anesthetics. The action of the latter has now been fairly well worked out and certain anesthetic drugs have been found to be without action on the blood sugar, with the result that the effect of operations can be accurately studied.

Griffiths (13) has found that these, especially if abdominal, tend to raise the blood sugar. In normal rabbits or cats under chloralose, it was found that stimulation of the central end of the crural or sciatic nerve caused an almost immediate increase in the blood sugar. Babkin (14) confirmed this result on the sciatic nerve in a decerebrate animal and also found concurrent changes in the blood pressure in the abdominal viscera. It has also been observed that removal of the submaxillary glands in dogs causes a rise in blood sugar (15).

The alimentary glycosuria which occurs in certain cases of pregnancy has been stated by Nothmann (16) to be of renal origin and not due to hepatic insufficiency, as evidenced by the blood sugar curve, which exhibits little or no deviation from the normal. During labour, if this be severe and prolonged, it has been found that an increase occurs in the maternal blood sugar (17).

In the previous review, work was quoted which showed that muscular exercise had an augmenting effect on the blood sugar level. Burger and Martens (18) tested 7 fasting subjects before and after 1 hour's gymnastics or boxing and found that in four cases there was no change. Two exhibited a fall and one a slight rise. From the evidence at present available, it seems hardly possible to come to a definite conclusion on this point.

It has been observed by Taya (19) that temperature exercises an effect on the blood sugar of normal rabbits. Sudden changes in the surrounding temperature have a definite effect. Cold produces a

rise (this confirms previous work) and warmth a rise followed by a fall to below normal.

Loss of sleep and mental fatigue appear to have no influence on the tolerance for sugar in either normal persons or diabetics (20).

In pigeons, it is stated that food and fasting do not alter the blood sugar concentration in adult birds, but the administration of food causes hyperglycemia, and fasting, hypoglycemia, in young which are being fed by their parents. It is also found that close confinement causes a reduction approximating to 30 per cent of the blood sugar, which rises to the normal when the birds are replaced in large cages (21). The latter author, in collaboration with Riddle and Spannuth (22), reports that birds with a low blood sugar content (0.142 per cent) were found to have large thyroids, suprarenals and gonads whereas in those with a higher level (0.181 per cent), these organs were of a smaller size.

Summarising the results of the experiments which have been referred to, it seems that the administration of glucose either by mouth or injection, invariably produces a rise in the blood sugar, whereas a normal meal may or may not do so. There appears to be some amount of conflict in the results of experiments dealing with the administration of levulose and lactose, but it is undoubtedly true that other sugars, in addition to glucose raise the blood sugar level.

V. THE EFFECT OF DRUGS, INORGANIC SALTS AND INTERNAL SECRETIONS ON GLYCEMIA. The effect of adrenalin has been studied by Lyman, Nicholls and McCann (1) who found that its administration caused a rise in the blood sugar concentration accompanied by an increase in heat production and a rise in the respiratory quotient. This was true, both for normals and diabetics. The preliminary administration of calcium chloride caused an increase in adrenalin hyperglycemia (2). This rise in the sugar content of the blood has been found by Gyorgy and Herzberg (3) to be followed by a hypoglycemia which may be prevented by the previous administration of bicarbonate, by irradiation with ultra-violet light and by inducing a condition of fever. The hypoglycemia may be increased by a preliminary administration of sal-ammoniac and these authors state that the equilibrium between acids and bases in the blood is only one of the factors influencing the result.

Hedon (4) reports that hyperglycemia followed the injection of 5 mgm. of adrenalin in two completely depancreatised dogs, during the first week of diabetes.

The hyperglycemic blood produced in this way is said by Eadie (5) to possess a lower rotatory power than normal blood.

The cause of adrenalin hyperglycemia appears to be a decrease in the blood diastase and an increase in the liver diastase so that, not only is more sugar freed from the liver and discharged into the blood stream, but there is less sugar destroyed in the latter, these factors combining to produce the hyperglycemia. Cohen (6) has stated that adrenalin causes a diminution of blood diastase accompanied by an increase in liver diastase and it is possible that the hyperglycemia in diabetes may be partly due to the same phenomena. Winter and Smith (7) state that the injection of adrenalin causes normal blood sugar to resemble diabetic sugar. The writer has observed a decrease in the diastase of diabetic blood, but sufficient cases have not yet been examined to warrant a definite statement on this point.

It is considered by Houssay and Lewis (8) that some mechanism exists in the body, which is activated by morphine and raises the blood sugar. This mechanism is hindered by cutting the splanchnic nerves, but if the vagi are cut it comes into play again. The same authors, in collaboration with Molinelli (9), report that the injection of morphine results in an increase in the secretion of adrenalin and it seems probable that the mechanism referred to is intimately connected with this increase in adrenalin secretion.

Hydrazine poisoning causes a fall in the blood sugar, accompanied by an increased blood concentration (10), and this blood sugar effect is confirmed by Bodansky (11), who reports that the same effect is produced by severe chloroform poisoning and by phosphorus intoxication. The latter author considers that the condition indicates severe liver damage, which is evidenced by the appearance of a preliminary hyperglycemia, the latter being especially well-marked in ill-nourished animals. It is stated by Tadenuma (12) that phenyl-hydrazine or saponin injected subcutaneously or intravenously have no effect on the blood sugar, except after a meal when a rise was observed to 0.25 to 0.35 per cent.

Other drugs which have been studied as regards their action on the blood sugar are urethane, which causes a rise (13), atophan and atoxyl, which also result in a rise (14), ergotamin, which brings about a decrease in normal rabbits by about 14 per cent (15), and isoamylethylbarbituric acid which has no effect. The latter is an anesthetic and, in view of its lack of influence on the blood sugar, has considerable

advantages in the study of operative conditions and in work on insulin; the dose in dogs is about 0.1 gram per kilo and in rabbits about 0.08 to 0.1 gram (16).

Caffeine, injected subcutaneously in doses of 0.15 to 0.25 gram per kilo body weight in dogs increases glycemia by 40 to 60 per cent, the maximum effect occurring two hours after injection (17).

McCullough (18) states that anaphylactic shock in dogs brought about an increase of blood sugar concentration amounting to a maximum of 0.25 to 0.27 per cent in as short a time as 10 to 30 minutes. This represented an average increase of 0.10 per cent above the initial concentration. It has also been shown by Laufberger (19) that intravenous injection of small amounts of protein in rabbits caused hyperglycemia and this author found that protein sensitisation was without effect in the reaction. Oral administration of protein has the same effect (20). Infections with organisms of the enteritidis-paratyphosus B group have been observed to cause a rise in blood sugar, associated with lesions in the pancreatic islet tissue, liver and kidney. A temporary immunisation was brought about against B. enteritidis with a corresponding fall in blood sugar concentration, the latter being correlated with a rise in temperature (21).

As regards the action of internal secretions, it has been demonstrated that parathyroid extract is without effect on the blood sugar (22), and that the injection of secretin causes a transient hyperglycemia, which is later followed by hypoglycemia. This is independent of pancreatic action and persists after removal of the gland (23).

Certain inorganic salts may exercise a considerable influence on the blood sugar. For example, the effect of intravenous injection of 10 cc. of 10 per cent calcium chloride was studied in twelve hospital patients by Barath (24), who found that, in six cases, it produced a slight rise, amounting to a maximum of 16 per cent. Potassium chloride produces a fall in blood sugar content (25).

The influence of phosphates on the blood sugar has attracted some amount of attention recently and experiments have been carried out by Fujimaki (26), who found that sodium dihydrogen phosphate increased the hyperglycemia caused by adrenalin, while the monohydrogen phosphate decreased it. The same author also found that the hyperglycemia induced by injections of glucose was augmented by the addition of the acid phosphate and reduced when glucose was administered with disodium phosphate. It is stated that phosphates

assist in the formation of glycogen from glucose. When the former is stored, the retained phosphorus is excreted, giving rise to the increased elimination of phosphorus occurring under such conditions.

There is some amount of conflict in the evidence regarding the effect of phosphates on the blood sugar in normal individuals. This may possibly be explained by the assumption that different animals react in different ways. Elias, Popescu-Inotesti and Radoslav (27) have demonstrated a difference in reaction between humans and rabbits and dogs. In the former, it is stated that both mono and disodium phosphate have no action, but in the cases of the animals referred to, the action is a depressant one on the blood sugar. Brugsch and Horsters (28) report that sodium hydrogen phosphate and phosphite lower the blood sugar but that tonophosphan, an organic phosphorus compound has no effect.

The results of Fujimaki, mentioned above, with monohydrogen phosphate have been confirmed by Elias and his colleagues (29), who found that adrenalin hyperglycemia was rendered less acute and lowered quicker by injections of this salt in dogs and rabbits. They also found that the same effect was seen in depancreatised dogs but that the effect on normal animals was nil.

It thus appears to be established that the action of the phosphate ion in this connection is confined to hyperglycemic conditions and that, in the normal animal, it is inactive in reducing the blood sugar.

VI. Glycemia in diseases other than diabetes. In view of the fact that diabetes is not the only condition in which pathological changes in the blood sugar concentration are found, the following notes may be of interest.

Holsti (1) found that, in cases of arthritis, a condition of alimentary hyperglycemia occurred which reached a maximum in one hour and did not attain the normal after a period of two hours. After treatment most of the patients improved and showed a normal tolerance for sugar.

That hyperglycemia may be associated with malignant disease of the gastro-intestinal tract has been demonstrated by Roffo and Rivarola (2) and the same authors (3) state that in cachexia there is hypoglycemia.

Daly, Pryde and Walker (4), in four cases of epilepsy, found abnormal variations in the blood sugar content, but they hesitate to associate these with the condition. They take the opportunity of mentioning that, so far as experimental methods are concerned, there is

no evidence that glucose is the only copper reducing and optically active substance present in the blood filtrate and that any attempt to estimate glucose polarimetrically is useless. They found, in some cases, that the solution was levo-rotatory and state that the copper reducing value of Winter and Smith's polarimetric solution exhibits irregular variations from day to day.

The blood sugar has been found to increase in infants, during severe dyspepsia (5).

Maranon, Carraxo and Soler (6) state that, in Addison's disease, hypoglycemia is always present and that the blood pressure falls concurrently with the blood sugar.

The work of Feinblatt (7) has already been mentioned in the section dealing with physiological glycemia. Of the eighty-one patients with hyperglycemia, only thirty-four were diabetic. Of the remainder, there were three cases each of hyperthyroidism, cerebral hemorrhage, chronic glomerular nephritis, chronic bronchitis and menopause and two each of rheumatic fever, neurosyphilis and syphilitic aortitis. It is pointed out that a prolonged follow-up study of these cases, which was not made, might have proved some of them to be diabetic and also that the hyperglycemia observed might have resulted from emotional disturbances, so that too much stress should not be laid on the correlation of this condition with the disease.

VII. Glycolysis in blood. In the previous review a considerable amount of conflict in the available experimental evidence was noted and while it was agreed that, under certain circumstances, a loss of sugar took place after the shedding of blood, there was no definite information forthcoming as to the causes of this glycolysis and very little as to its degree and the conditions favouring it. Since 1923, a considerable quantity of new work has been published, but there is still some conflict in results. The fresh evidence which is available includes the observations of Aibara (1), who found that the serum of dog's blood which had been freed from all formed elements did not undergo any loss of sugar. This author also states that sodium fluoride and potassium oxalate prevent glycolysis, but that sodium citrate has no such action. Fuentes and Rubino (2) report that small quantities of calcium chloride increase glycolysis while, if this salt is added in large amounts, it exercises an inhibitory action. The effect of mercuric chloride in a concentration of 0.5 gram per 100 cc. is also inhibitory, but a smaller percentage (below 0.05 gram per 100 cc.), causes an increase in glycolysis.

The effect of temperature has been studied by incubating the blood

at 37° to 38°C. In bird's blood treated in this way, Ruter (3) has found practically no loss of sugar during a period of three hours. Bierry, Rathery and Kourilsky (4) find that a temperature of 38° maintained for 24 to 48 hours causes a diminution, sometimes to zero, in the free sugar. The protein sugar according to these authors, diminishes more slowly and there may even be an initial increase. Bierry and Moquet (5) state that the decrease in the free sugar is accompanied by an increase in the inorganic phosphorus of the blood, after a small initial fall. This, they consider, points to the formation of a hexose-phosphorus compound. Glycolysis in bird's blood is attributed by Denecke and Eimer (6) to action of the young blood corpuscles.

Elias, Guederann and Kornfeld (7) state that the injection of phosphates does not increase glycolysis, so that it is impossible to explain the decrease in the sugar of diabetic blood after administration of phosphates by an increase in glycolysis.

It has been suggested that, in diabetic conditions, glycolysis is less active. Thalhimer and Perry (8) quote figures which show, in nine cases of diabetes, percentage decreases in 24 hours varying between 9 and 69, with an average of 48.3. In six normals after administration of glucose, the decrease in the same period varied between 35 and 84 per cent, the average being 55.4. It seems, in the writer's opinion, hardly wise to draw definite conclusions on such a small number of cases. Denis and Giles (9) also state that glycolysis is much less in diabetic than in normal blood. On the other hand, Burger (10) states that only in some diabetics was there a diminution in glycolysis and this author correlates the loss of sugar with the degree of acidosis, supporting this by the statement that the addition of β-oxybutyric acid retards glycolysis and that acetone in small quantities accelerates and in large amounts reduces it.

The theory that glycolysis is reduced in diabetic blood is definitely opposed by Tolstoi (11), Mauriac and Aubertin (12) and Cajori and Crouter (13), none of whom could find any evidence to this effect.

This question, in some of its branches, has been studied by the writer and details of his experiments appear elsewhere. They may be summarized as follows.

Sterile oxalated blood loses practically all its sugar in ten days at room temperature. The greatest loss appears to take place in the second 24 hours. The loss of sugar in venous blood, kept at room

temperature, after 24 hours averaged 3 per cent less and after 48 hours 2.5 per cent less than that in blood obtained from a finger prick.

Sugar disappears from oxalated sheep's blood in amounts averaging 13.9 per cent in the first 24 hours and 25.2 per cent in the first 48 hours. After 72 hours, between 25 and 49 per cent and after 96 hours between 52.5 and 65.5 per cent was lost. The statements of other observers that glycolysis is almost absent in sheep's blood is not confirmed. In 24 hours, the amount lost is comparable to that in human blood, but after 48 hours, the loss is only about half that seen in human blood. The fluctuations occurring in the loss of sugar, which apparently bore no relation to the original sugar content of the blood, gave rise to the possibility of a surface phenomenon being involved and it was decided to measure off accurately two equal volumes of blood for estimation after 24 and 48 hours, instead of leaving the blood in one vessel for the whole period. This, it was considered, would tend to neutralise any error which might possibly be introduced through the comparison of unequal volumes of blood. Accordingly, the blood under investigation was divided into three portions, two of which were of equal volume, so that approximately the same relative surface of each specimen was exposed to the air. The first estimation was made from the main bulk of the blood and the other two, at the end of 24 and 48 hours respectively from amounts of 0.5 cc., kept in different vessels of the same capacity. Under these conditions smaller fluctuations were observed and the average loss was about 2 per cent less at the end of both periods. The number of specimens examined (14) is, however, too small to warrant any definite conclusions as to the relation between the surface exposed to the atmosphere and the amount and rate of glycolysis.

Seven cases of diabetic blood were examined with no evidence that glycolysis was less than in normal blood.

As regards the effect of different anticoagulants, it appears that sodium fluoride preserves the sugar content better than sodium citrate, but for the first 24 hours neither of these is as effective as potassium oxalate. In 48 hours, the disappearance seems to be least with fluoride. The sugar content of protein-free filtrates does not remain constant even for 24 hours. An average of about 1 per cent disappears in this time, whether the filtrate is prepared by Maclean's method or by that of Folin and Wu and the loss in 48 hours is approximately 3 per cent. A greater loss is almost invariably seen with Maclean's method. It is suggested that small amounts of protein in the filtrate, which it is

very difficult to avoid, may exercise an influence in determining the degree and rate of glycolysis.

As regards the fate of the destroyed sugar, nothing definite can be said, although as a matter of interest, it may be mentioned that the author has recently isolated both saccharic and mucic acids from a sample of sheep's blood which had been standing in the laboratory for some days. This indicates an oxidation process upon lines which have hitherto been little considered, but whether this is the result of bacterial action or of a specific enzyme remains to be decided and work is now in progress which it is hoped may elucidate this problem and throw some light on the normal path of sugar metabolism in the body.

VIII. Experimental diabetes. Histological changes in the islets of Langerhans. The relation of the pancreas and the liver to carbohydrate metabolism. The experimental evidence which was available on the occasion of the previous review was somewhat contradictory and it was difficult to draw any satisfactory conclusions. However, there is no doubt that the study of diabetes by means of experimentally inducing the disease in animals has led to discoveries of considerable importance, even if, at present, the findings of different workers cannot be reconciled. It has been established that both qualitative and quantitative changes take place in the islets of Langerhans as a result of diabetes, whatever the precise significance of these may be and there seems no doubt that a close and intimate relationship exists between the pancreas and the liver in carbohydrate metabolism. The work which has been carried out since 1923 is considerable and a large amount of fresh evidence is forthcoming which will now be summarised.

In connection with phloridzin poisoning, which, as is well known, is one of the methods for producing experimental diabetes, Nash and Benedict (1) suggest that this drug exerts an action which not only affects the permeability of the kidney tissue to the blood sugar, but also produces an intrinsic impairment of the body's mechanism for effecting the combustion of sugar. Hornemann (2) finds that if levulose is administered to phloridzinised dogs during fasting, there is a considerable increase in the blood sugar and that both with levulose and glucose the deposition of glycogen is small, the utilisation of these sugars being diminished as compared with normal dogs. Nash and Benedict (3) do not agree with the view of Ringer, that phloridzin injures the pancreas and thereby prevents the production of the antidiabetic

hormone, since they have been able to isolate insulin from the pancreas of phloridzinised dogs. It may be pointed out that these views are not necessarily incompatible.

Allen (4) states that phloridzin does not injure the islets of Langerhans and also that removal of most of the adrenal tissue in dogs produced no effect on the carbohydrate tolerance of these animals. In dogs, partially pancreatectomised, the administration of adrenalin failed to produce diabetes, but in animals with the disease well developed this drug greatly increased the excretion of sugar and of nitrogen and Allen considers that this fact explains the abnormal susceptibility of diabetic tissue to breakdown.

The interrelation of the maternal and the foetal pancreas has been studied by Aron, Stultz and Simon (5), who find that extirpation of the maternal pancreas does not produce diabetes, provided that the fetal pancreas is sufficiently developed, and Aron (6) also considers that there is a decided distinction between the glycogenic functions of the liver and the mechanism for the regulation of the blood sugar concentration.

It must be borne in mind that the results which are available regarding the operation of pancreatectomy indicate that considerable individual variations exist in the reaction of different animals to this procedure. This has been commented on by Mauriac and Aubertin (7) and introduces a considerable obstacle to the correct interpretation of results obtained in this way.

After bilateral ligature of the renal vessels or of the renal veins alone Morita (8) has demonstrated that a transitory hyperglycemia appears immediately after the operation. This is followed by a secondary rise which occurs some days after and it is further shown that the first effect is not seen in bilaterally splanchnicotomised animals, but that the second occurs independently of all nerve section. This indicates both a central and a peripheral influence in hyperglycemia.

The nature and site of the central control of glycemia has been investigated by Camus, Gournay and LeGrand (9) who produced experimental diabetes by introducing a capillary tube filled with fatty acids into the pituitary region in rabbits. Eighteen complete experiments were performed and in ten positive cases the lesion was localised in the tuber cinereum. In those cases which were negative the lesion was always found at some point near the tuber. Studied histologically by means of serial sections, it was found that the paraventricular nucleus was constantly and the nucleus of the tuber frequently affected. The

authors consider that the close contact of these centres suggests that one nucleus may produce polyuria and the other glycosuria. A lesion of the tuber causes a more lasting and a graver diabetes than the Claud Bernard sugar puncture.

A histological study of the pancreas of the frog, carried out by Aron and Schwartz (10), indicates that distinct seasonal variations exist. These are correlated with the glycogen content of the liver, which varies from 12 to 16 per cent in the winter to 2 to 6 per cent in the summer. In mammals, it is stated by Cori and Pucher (11) that the free sugar content of the liver tissue, even in starved animals, amounts to 0.3 to 0.4 per cent. After administration of glucose, the free sugar rises and this is then followed by an increase in the glycogen content. The administration of adrenalin results in a large rise in the free liver sugar (to over 1 per cent) and a reduction in the glycogen. The decrease in free sugar which occurs under the influence of the pancreatic internal secretion is accompanied by a small rise in the glycogen content.

It is found that withholding cell salts from the dietary causes a slight diminution in the liver glycogen and a slight increase in the blood sugar of rats (12). The influence of isolated salts has been studied and it is found that potassium salts have no appreciable effect. Calcium produces a slight increase in the blood sugar, but has no influence on the liver glycogen. The addition of phosphates to a low salt diet reduces the blood sugar to normal but has no effect on the liver glycogen.

Albertoni (13) states that, in order to test the liver function, it is only necessary to determine the blood sugar concentration about 1 hour after the ingestion of glucose. The author finds that, in some cases of cirrhosis, hyperglycemia occurs without glycosuria after glucose administration and that this condition also occurs in various other diseases. It is considered that the fact is well established that, in normal individuals, the liver performs an almost complete protective and regulating function in sugar metabolism.

IX. Diabetes. Treatment from the physiological aspect. Glucose tolerance tests. It is now generally recognised that the treatment of diabetes by insulin must be supplemented by a certain amount of dietary restriction in addition. Under these circumstances insulin is a most valuable therapeutic agent but its use without dietary restriction is to be deprecated, since no opportunity is then given for rest to the overstrained islet tissue. It appears to be established beyond reasonable question that the supplying of the pancreatic internal secretion by means of insulin replaces the lack of this in the dia-

betic individual, although of course if the islet tissue has become permanently damaged, the treatment can only be palliative. Evidence is forthcoming, nevertheless, that under certain conditions insulin treatment may result in an actual regeneration of the damaged islet tissue:

The great weight of evidence which the past few years have produced must be considered definitely to refute a theory recently advanced by Oertel (1), in which this author questions the independence of the islets of Langerhans as organs of internal secretion, essential for sugar combustion, although there may still be some uncertainty as to the exact mechanism of the relationship of the pancreas to sugar metabolism.

The two theories of diabetes which are held at the present time are outlined by Winter and Smith (2) who, it will be remembered, have attacked the problem from the point of view of the nature of the blood sugar. One theory states that the disease is the result of the overproduction of sugar and regards the pancreas solely as an inhibitory mechanism, attributing the ill-effects of diabetes to the toxic effect produced on the tissue cells by the high concentration of sugar. The second view is that diabetes is due to the progressive inability of the tissues to utilise sugar and that in some way the internal secretion of the pancreas enables the tissue cells to deal with the sugar by oxidation. Winter and Smith consider that the latter theory is the more probable and they adduce evidence pointing to the formation of a reactive sugar in the body during diabetes which lends powerful support to this view. They hold the opinion that there are two types of sugar concerned, one normal and the other abnormal, the normal being removed by the cells and oxidised. The abnormal type, these authors consider, cannot be oxidised and is excreted.

An attempt to classify diabetes mellitus on the basis of blood pressure has been made by St. Lorant and Adler (3). These workers have demonstrated that in cases of high blood pressure there is a corresponding increase in the blood sugar concentration and that the maximum of sugar excretion coincides with the minimum of blood pressure.

Allen (4) suggests that ketosis is not only the result of the quantitative relation of the glucose and fatty acids metabolised, but that a biological factor is concerned.

Enroth (5) has found that the hypermetropia found in some diabetics is due to a transitory change in eye refraction, which is attributed to the incident acidosis. It is shown that acetone and assumed that diacetic acid tend to accumulate in the aqueous humor of normal eyes in greater concentration than in the blood and it is suggested that, al-

though the acetone bodies cannot directly affect refraction, through osmotic changes an alteration in the lens is established which manifests itself as hypermetropia.

To return to some points in the treatment of diabetes, Kahn (6) has found that an artificially prepared fat (glyceryl ester of margaric acid) is absorbed to the extent of 95 per cent and that administration of this to phloridzinised dogs caused a marked increase in the elimination of glucose in the urine. This indicates that it is broken down to propionic acid and then converted into glucose as supposed by Ringer. Kahn states that this odd-carbon fat may be safely administered to diabetics in amounts yielding as much as 1000 calories per day. It thus follows that, if the patient's diet has been increased by means of insulin to 1500 calories, the additional 1000 calories required to provide a maintenance diet can be added by the administration of this fat.

Lundin (7) has made some similar observations on odd-carbon fats and states that 100 grams of natural fat can be replaced by an equivalent weight of the odd-carbon fat. Ketosis resulting from a high fat diet (177 grams fat plus 20 grams carbohydrate), under such circumstances is replaced by an acidosis, due to lactic or pyruvic acids. If the carbohydrate is increased by 50 grams this disappears and it is thus apparent that odd-carbon fatty acids require carbohydrate for their complete metabolism.

In the diagnosis and treatment of diabetes, the glucose tolerance test is very valuable, since it is simple to carry out and at the same time offers information as to the reaction of the body to glucose which can be obtained in no other way. The technique of the test involves merely the administration of a dose of glucose and the estimation of the blood sugar concentration at different periods, immediately before and after. The test should be carried out in the morning and the patient should have no breakfast. A sample of blood is withdrawn for estimation before the dose of glucose is given and the bladder is emptied. The amount of glycosuria, if any, is determined and 50 grams of glucose dissolved in about 150 cc. of water are administered. Further samples of blood are taken at intervals of $\frac{1}{2}$, 1, $1\frac{1}{2}$ and 2 hours and the sugar content estimated. From these data a curve is plotted, from the general appearance of which information of considerable importance can be obtained. In normal persons, the rise of blood sugar following the ingestion of glucose is neither as high nor as long continued as that seen in diabetics.

The height of the renal threshold may be studied in the same way, by making, in addition, estimations of the concentration of sugar in the urine.

A simple food tolerance test has been devised by Brill (8), in which a normal meal is substituted for a dose of glucose. It is stated that this is more agreeable to the patient and that it avoids the danger of overtaxing a weakened function by a large quantity of pure glucose. The meal consists of bread, butter, cereals, eggs, sugar, coffee and cream, amounting to approximately 100 grams of carbohydrate, 26 grams of protein, 27 grams of fat and about 760 calories. Only two estimations of blood sugar are made, one before the meal and the other $1\frac{1}{2}$ hours after. In a normal individual, $1\frac{1}{2}$ hours after the meal, the blood sugar should be within 10 mgm. of the fasting level. In diabetics, the second level is invariably considerably higher, in mild cases averaging 25 to 36 mgm., in moderately severe 40 to 50 mgm. and in severe cases over 200 mgm. higher. Insofar as it only involves two sugar estimations, this test appears simpler than the other mentioned above, which was devised by Maclean (9), but the author has had no personal experience of it.

X. INSULIN. *Its preparation and chemical properties.* The story of insulin has such a recent beginning that it is no doubt familiar to most of the readers of this review. It was touched upon in the previous summary of the subject two years ago, but since then, as is not surprising, an enormous amount of work has been done on the subject and it is difficult to do justice in a review such as this to the whole of the literature. However, an attempt will be made to summarise the most important evidence which has become available with respect to this substance, its properties and functions. The present section of this review will deal with those conceptions of the action of insulin which have been evolved as a result of experimental evidence, together with some notes on its preparation and chemical properties. Its physiological effects will be left to a later section.

While the first impression which arose as to the action of insulin was a general one, in which it was assumed that its action in lowering the concentration of sugar in the blood was the beginning and end of its function, later work points to the fact that this substance has two functions. These have been detailed by Gabbe (1), who states that: *a*, it increases the consumption of carbohydrate in the tissues, in the absence of new formation of sugar from other substances, and *b*, it prevents the transformation of glycogen into combustible sugar. This author's mean-

ing does not appear quite clear but the two function theory is supported by Fisher (2), who states that insulin does not represent the whole of the pancreatic hormone, and by Ambard, Schmidt and Arnovlyevitch (3), who report that there is no slowing down of glycogenolysis during the action of insulin, which fact they take to indicate the presence of two hormones. These authors suggest that hyperglycemia inhibits the secretion of insulin, whereas Sevringham and Smith (4) state the direct opposite. They find that glycogen is stored as an immediate result and that thereby glucose is utilized by the tissues. The insulin produced outlasts the need for it and hypoglycemia is induced, which stimulates the glycogenetic mechanism and the blood sugar returns to the normal fasting level.

It must be pointed out that, while these theories are ingenious and have much to recommend them, there is still a lack of experimental evidence sufficiently definite to justify the unreserved acceptance of one or other of them.

Allen (5) considers that the insulin requirement of the organism is governed by all the elements of the diet and is related quantitatively to the body mass. This author also holds the view that insulin has two functions, one anabolic and the other katabolic.

The preparation of insulin. Numerous modifications of the original methods have been proposed and, in the last review, Dudley's modification of Collip's method was described in some detail. Clough and Murlin (6) consider that the most efficient method is one of gravity perfusion with 0.2 per cent hydrochloric acid at a temperature between 37 and 45°C. and a pressure of 120 mm. of mercury for one hour. In view of the expense involved in the original methods, owing to the large amount of alcohol required, many workers have turned their attention to the possibility of securing a satisfactory extraction with acid and, in addition to those workers mentioned above, Dodds and Dickens (7), Sornogyi, Doisy and Shaffer (8), Penau (9) and Boivrin, Oddo and Chosson (10) have devised methods employing an acid extractive. Dudley and Starling (11) find that extraction of minced pancreas in an alkaline medium increases the yield fivefold.

An important feature in the preparation of insulin is the necessity of eliminating the toxic fraction which is sometimes present. Fisher (12) precipitated two fractions from the final filtrate with 95 per cent alcohol. Both were soluble in water, one contained protein and was toxic, exerting a hyperglycemic action and this, the author concludes, probably causes the local irritation seen sometimes with insulin injec-

tions. The other fraction only contained a trace of protein and was actively hypoglycemic.

Penau claims that his method produces insulin which does not cause harmful secondary reactions.

Sammartino (13) states that the occurrence of small quantities of diastase and catalase are incidental to the preparation of insulin.

It is unnecessary to go into further detail in connection with this branch of the subject. Enough has been said to show that the problem of securing the highest efficiency in the final product is one which is receiving considerable attention and at the present time, there is no doubt that the insulin which is on the market is for the most part quite satisfactory.

The chemical properties of insulin. These have been worked out in some detail during the past two years, and whereas there appears to be some amount of contradiction in evidence, it will generally be found that this can be explained by the different methods of manufacture which have been adopted. It must be confessed that the nature of insulin has not yet been determined and there is, at present, some conflict in the results of researches on this problem. In this respect and in the difficulty of obtaining it in an absolutely pure state, insulin resembles the vitamins, but it is to be hoped that the task of elucidating its chemical structure will not prove so difficult as in the case of the latter bodies.

The chemical properties of insulin, so far as they have been ascertained by experiment, are now detailed.

Insulin hydrochloride obtained from the crude insulin prepared by Collip's method contains sulphur but no phosphorus, gives the biuret and Pauly's tests, is destroyed by pepsin and trypsin and by alkaline solutions, but is fairly stable in acid solution. Dudley (14) considers that it is of the nature of a complex protein derivative. Witzemann and Liveshis (15) find that insulin is completely inactivated by proteolytic enzymes, and by alkalis. The action is irreversible with sodium and potassium, but, in the case of ammonia, immediate acidification may restore the activity. Its destruction by proteolytic enzymes is confirmed by Shonle and Waldo (16) and, as far as trypsin is concerned, by Epstein and Rosenthal (17). The latter authors find that the insulin only is inactivated and that the action is not mutual, i.e., the trypsin remains active. It occurs only at a hydrogen ion concentration above 4.6. Between 4.6 and 6.0, a precipitate is formed which is soluble in more alkali or in acid solutions. The insulin remains inac-

tive in the former but fully active in the latter. The authors conclude that alkali alone does not inactivate insulin and that trypsin only inactivates it when it is itself active.

Further properties of insulin have been reported by Moloney and Findlay (18) who state that it is insoluble in ether, chloroform, petroleum ether, ethyl benzoate, methyl salicylate, carbon tetrachloride and turpentine. It is completely soluble in glacial acetic acid, cresols, phenol and formamide.

It is slightly soluble in acidified absolute alcohol but is precipitated from solution by dilute ferric chloride and by half saturation with ammonium sulphate, also by picric acid. Barium salts of weak acids partly precipitate it and dilute solutions of copper sulphate dissolve it.

It is completely destroyed at room temperature by sodium hydroxide, at a reaction equivalent to pH 14, in two hours and at pH 13 in 16 hours, but is relatively stable to mild oxidation. Its strength appears to be best preserved in a fairly acid medium, e.g., 0.06 to 0.08 per cent HCl (19).

As has already been indicated, the problem of its constitution has not yet been solved and there appears to be some doubt as to whether it is a protein derivative or not. Widmark (20) considers that it may be an albumose, Shonle and Waldo (16) that it is a mixture of proteoses and Fenger and Wilson (21) that it is a protein substance of some sort. Doisy and Webber (22) find that the insulin purified by fractional precipitation from alcoholic solutions gives a biuret reaction corresponding to an equivalent weight of peptone, whereas Piper, Allen and Murlin (23) have produced a potent preparation giving none of the usual protein reactions.

Ganassini (24) states that insulin prepared by Macleod's method contains a base of the cholin group and a principle which is highly active and which probably belongs to the jecorin group. This author also finds that the insulin prepared by Collip's method appears to consist of jecorin only.

The composition of insulin sulphate prepared by Cruto is stated by this author to be as follows: C:47.73, H:7.27, N:14.53, S:1.73, O:22.84, H_2SO_4:5.9.

Finally, Berkeley (25) has postulated a hypothesis which indicates a connection between the production of insulin and the high concentration of pentose compounds which is found in islet tissue, so that, until further and more definite evidence is forthcoming, its constitution

remains doubtful, although it certainly appears, at present, to be of the nature of a protein derivative.

XI. The sources of insulin and insulin-like substances. Although, at the time of the discovery of insulin, the islets of Langerhans of the mammalian pancreas were regarded as the only source of this product, it appears, as the result of more recent researches, that insulin or substances having a similar action may be derived from many other tissues, not only in the mammalian body, but also in fish and from various vegetable sources. The work of Collip (1) who is one of the pioneers of this subject deserves particular notice and this author claims to have been the first to discover the existence of a principle in certain vegetable extracts, which caused a reduction in blood sugar concentration. Collip named this substance "glucokinin." It was first found in an extract made from onions. Shortly afterwards, Best and Scott (2) reported that preparations manufactured from a 70 per cent alcoholic extract of potatoes, rice, wheat, beetroot and celery, upon injection into normal rabbits, produced a fall in the blood sugar. These authors, in collaboration with Banting (3) also found an insulin-like substance in the blood of normal rabbits and dogs, in hypoglycemic rabbits and in ox blood and that this substance was reduced in amount in the blood of diabetic dogs. Similar substances have been found in yeast extract, the amount varying widely with the particular variety of yeast used (4), in oranges, lemons and grape fruit (5), from dried beans (6) and from an alcoholic extract of oat bran. The latter, according to the discoverers, Boden, Neukirch and Wankell (7) caused a fall in blood sugar in $1\frac{1}{2}$ to 2 hours with a dose relatively small compared with Collip's glucokinin.

Bacteria grown on an insulin-free medium are stated by Little, Levine and Best (8) to yield a substance which lowers the blood sugar of rabbits; and Hutchinson, Smith and Winter (4) also report the isolation of an insulin-like substance from a coliform bacillus. Brugsch and Horsters (9) state that the insulin material obtained from yeast differs from that originating in the pancreas and these authors also investigated peas and lupin seeds, with a negative result.

Ashby (10) has prepared substances from the kidney, spleen and skeletal muscle in the ox and dog, which lower the blood sugar of rabbits, but the effect of these is not so powerful as that of insulin proper. Nevertheless, the effect of the kidney extract lasted for 24 hours or more. The same author also reports that extracts from the tissues of depancreatised dogs were inactive.

This work is, for the most part, confirmed by Best, Smith and Scott (11), who investigated the thymus, the liver and the skeletal muscle and also blood in the calf and the dog. They report that less of the insulin-like substance is found in the tissues of depancreatised dogs and less still in a dog under ether anesthesia. Baker, Dickens and Dodds (12) state that the insulin found in the kidney and spleen appears to be similar to that from the pancreas and that the organs of a diabetic dog dying in coma contained appreciable amounts of this product.

As regards the presence of insulin in the tissues of fish, McCormick and Noble (13) state that it occurs in all fish, in amounts comparable to those obtained from mammals and they draw attention to the lessened cost of production of insulin obtained in this way, emphasising that the product has the same reaction in diabetes as that obtained from mammalian pancreas. A further plea for the production of insulin from fish is put forward by Dudley (14), who states that the yield from the islet tissue of codfish, treated by direct fixation in picric acid, extraction of the picrate with watery acetone and conversion into the soluble hydrochloride, amounts to ten times as much as that from mammalian pancreas.

The relative merits of insulin prepared in the different ways indicated have not yet been fully tested, but there seems to be no doubt that this substance may be equally well derived from the several sources mentioned and it will probable lie with those responsible for its commercial development to devise the cheapest and, at the same time, the most efficient method of producing it.

XII. The physiological effects of insulin. Up to the time of publication of the previous review, the chief points in connection with the physiological action of insulin which had been reported were its lowering action on the blood sugar and the possibility of the occurrence of certain reactions due to overdosage. In addition, some amount of antagonism between pituitary extract and insulin had been noted. Since 1923, the volume of literature dealing with the physiological effects of insulin alone exceeds one hundred papers, so that the interest which the study of this subject has aroused is obvious. Some of the more important work will now be outlined and, for the sake of convenience, this is divided up under a number of subtitles.

a. General. It has been found that, in the insulin which is prepared from vegetable extracts, two fractions occur. One causes hypoglycemia and the other hyperglycemia (1). It is also stated that animals

react differently to the same dose of insulin and the variations which occur in the resulting hypogylycemia are not proportional to the dose. It is concluded by Desgrez, Bierry and Rathery (2) that there is an optimum dose for each individual. The action of insulin in different animals has also been studied by Sordelli, Houssay and Mazocco (3), who found considerable variations in the amount necessary to produce a given degree of hypoglycemia and also in the lethal dose. Young animals were more sensitive than adults.

Insulin hypoglycemia has been found by Bodansky and Simpson (4) to be of greater degree in lean than in fat subjects, and, from experiments carried out by perfusion of frogs' kidneys with glucose solutions after the administration of insulin, v. Creveld and v. Dam (5) consider that satisfactory proof is afforded that insulin exerts a direct effect on the kidneys. Huxley and Fulton (6) conclude, also from experiments on frogs, that insulin acts only as rapidly as the tissues, by virtue of their metabolic rate, are capable of being affected by it. The general reactions to insulin have been studied in tadpoles by Kroszcznski (7) who found that immersion in an insulin solution for 4 to 5 hours caused excitement. During the following 12 to 16 hours movement gradually decreased and, latterly, there was hardly any response to mechanical stimulation. This was followed by failure in equilibration and convulsive tremblings, finally, after 24 hours, resulting in coma and death. The action of glucose was found to inhibit the effect of insulin, as were also hypophysis extract and atropine. Pilocarpine and physostigmind produced an augmenting effect.

In pigeons, the maximum reduction in blood sugar following the injection of insulin, took place in less than two hours. The birds exhibited considerable variation as regards the time of return to the normal. In doves, however, more constant results were obtained and the normal was regained in 4 hours. Larger doses produced much lower sugar values and the time of return to normal was considerably protracted, extending in some cases to 6 hours (8). Riddle (9) has also found that, in most cases, insulin suppressed ovulation in pigeons. At the period of ovulation there is normally a marked hypertrophy of the adrenals and an increase in the blood sugar. By the lowering action on the blood sugar, ovulation is suppressed. It is stated that pigeons stand relatively enormous doses of insulin and, in this connection, Riddle, Honeywell and Fisher (10) have found that, after one large dose, or repeated doses, an enlargement of the suprarenals occurs,

which appears to affect the cortex particularly. The most marked effect was seen in birds with the largest suprarenals and the authors suggest that the production of adrenalin is increased by insulin.

The optical activity of the blood of normal rabbits was found by Eadie (11) to be unaltered by the administration of insulin except in one of the cases examined. In dogs, it was found that there was an increase on consecutive days, until the reading coincided with that calculated from the reducing power of the blood. It was further found that insulin and adrenalin, administered together, gave a rise in two cases which was greater than that calculated from the reducing power.

Winter and Smith (12) state that the copper-reducing power of the blood of normal rabbits becomes smaller and smaller, following the injection of insulin and that, when the animal is nearing the period of convulsions, it is quite absent, but, nevertheless, the blood contains a dextro-rotatory substance of a carbohydrate nature. The fall of blood sugar which occurs as a result of the administration of insulin is considered by Burn and Dale (13), from experiments on eviscerated and decapitated cats, to be due to the action of the heart, lungs and muscles and, according to Dudley (14), it does not appear as glycogen, nor is it converted into fat. The stores of glycogen tend to disappear. Nitzescu and Popescu-Inotesti (15) state that, coincident with the fall in free sugar, there is a rise in protein sugar and the same authors (16) explain the increased capacity of the organism to utilise sugar after the administration of insulin on the basis that a temporary linkage of some of the free sugar to protein takes place. The fall in blood sugar after insulin is regular, proceeds in stages and is accompanied by a rise in temperature. Before death in convulsions, it is stated that there may be a slight rise (17).

In seven experiments carried out by Frank, Nothmann and Wagner (18), it was found that, after insulin, the fall of sugar was at first greater in venous than in arterial blood but, after a short time, the amount was the same. These authors consider that the essential section is exerted on the muscles and that it enables them to remove more sugar from the blood than normally, converting it into some form which can be readily catabolised.

The view that a direct glycolysis does not occur as a result of insulin is supported by Ducceschi (19).

In sheep, it is found that convulsions do not occur, even when the blood sugar is lowered to 0.03 per cent and that the effect of insulin

appears to be not so much on the intensity as on the duration of the hypoglycemia (20).

In cold-blooded animals, including fish and crocodiles, hypoglycemia as a result of insulin only occurs after considerable delay, but the same signs of excitability, convulsions and disorders of equilibration which are characteristic of mammals are exhibited (21). In crayfish and butterfly larvae, according to Hemmingsen (22), insulin appears to have no effect on the rate of disappearance of injected sugar, while in the heart-lung preparation, Plattner (23) reports that insulin added to the blood does not increase the rate of disappearance of the sugar, unless the heart beat is quickened. It is then found that the sugar disappears in the same ratio as the heart rate increases.

b. The effect of insulin on the blood cells, blood pressure and blood volume. Nitzescu and Mangiuca (24) state that insulin tends to produce a leucopenia which is quickly succeeded by a distinct leucocytosis. No change appears to occur in the differential count and the same results were seen in normal and depancreatised animals and in cases of human diabetes. These findings are not confirmed by Mazzocco and Morera (25) who observed no modification of the number of red or white cells or of the percentage of leucocytes following the injection of insulin in dogs. On the other hand, Levine and Kolars (26) state that insulin increases both the red and white cells, that of the latter being always greater. The rise in the number of red cells was proportional to the decrease in blood sugar. The mild leucocytosis observed is attributed partly to an increase in the blood concentration and partly to protein and other substances in the insulin preparation. The anhydremia appears to rise in proportion as the blood sugar falls, but there seems to be no relation between its amount and the size of the dose of insulin.

Drabkin and Edwards (27) have also observed an increase in the blood concentration following insulin in dogs (20 units per kilo), with an accompanying small drop in the blood pressure. If the anhydremia continues to increase, the blood pressure falls rapidly and the animal dies. After a dose of 50 units of insulin, the blood volume in a diabetic patient was reduced by 11.6 per cent two hours after administration and, in a normal individual, 40 units brought about a reduction of 9.8 per cent in one hour. Accompanying the decrease in blood volume was a diminution in the ratio plasma:corpuscles (28).

Widal, Abrami, Weill and Laudat (29) observe that hydremia (estimated by determining the refractive index of the serum) is generally

produced by insulin injections. It is characterised by sudden onset, intensity and duration and may lead to edema. It is not related to the state of hydration of the tissues before treatment, nor, invariably, to the metabolic response to treatment. Kay and Smith (30) state that, so far as rabbits are concerned, dilution of the blood is not a specific result of the injection of insulin and it will be seen, from the above notes, that the evidence on the question of hydremia is somewhat contradictory.

With further reference to the subject of blood pressure following the administration of insulin, it has been found by Edwards and Page (31) that, after a dose of 30 units in dogs, causing a fall in blood sugar to 0.05 to 0.012 per cent, the blood pressure fell 20 to 40 mm. Hg in the first two hours. It then either remained steady or showed a much more rapid fall, correlated with weakening of the heart beat, following an increase in rate and peripheral dilatation. The force of the heart beat was improved by the intravenous injection of glucose.

Klemperer and Strisower (32), Sammartino and Liotta (33) and Garrelon and Santenoise (34) also confirm the lowering action of insulin on the blood pressure and the latter authors also state that the oculo-cardiac reflex is augmented and the heart and respiration slowed. The presence of albumin in the insulin preparation may modify the effect which is considered to be due primarily to powerful excitation of the vagus.

c. Effect of insulin on reducing power of cerebro-spinal fluid. The only information on this point which is available is reported by Kasahara and Uetani (35), who state that the subcutaneous injection of insulin causes a decrease in the reducing power of C-S fluid.

d. The relation of insulin to phosphorus. It has been found by Kay and Robison (36) that insulin causes an increase in the organic phosphorus esters of the corpuscles and these authors consider that nearly 40 per cent of the sugar which disappears may be accounted for in the form of hexose phosphate. It is suggested by Sokhey and Allen (37) that phosphate is required for the metabolism of sugar and that, after temporary use, it is released causing the slight increase in the excretion of phosphates which follows the fall observed after insulin administration. The decrease in blood phosphate is confirmed by several workers, notably Wigglesworth, Woodrow, Smith and Winter (38), Perlzweig, Latham and Keefer (39), Briggs, Koechig, Doisy and Webber (40), and Blatherwick, Bell and Hill (42). The latter authors also support

the views of Kay and Robison in respect to the formation of a hexose phosphate.

Savino (42) states that the diminution in phosphate during insulin hypoglycemia cannot be due to insulin itself, since it occurs when glucose is injected either alone or together with insulin and it has been found that the effect of adrenalin is not antagonistic to that of insulin as regards the metabolism of phosphorus (43).

e. Effect of insulin on the blood lipoids, etc. Hartmann (44) states that, in three out of four cases, the administration of insulin slightly lowered the total lipoids of the blood and it is also reported by Fonseca (45) that insulin reduced the fat, acetone and β-hydroxybutyric acid in the blood of two diabetics to normal values. In a healthy individual it also caused a reduction in the blood fat.

In experimental diabetes in dogs and in the natural form in man, the blood cholesterol is greatly increased. Nitzescu, Popescu-Inotesti and Cadariu (46) report that a considerable reduction is brought about in such cases by insulin, but that this has no effect in normal animals.

f. The relation between the liver and the effect of insulin. Mann and Magath (47) consider that the liver is unnecessary for the hypoglycemic action of insulin. There is no evidence, according to McCormick and Macleod (48), that insulin causes any change in glycogen formation, except possibly in the heart. In the liver, the deposition is less and this may be related to muscular excitability, which is one of the early results of overdosage. In contradiction to this, it is stated by Collazo, Handel and Rubino (49) that insulin causes an increase in glycogen in both liver and muscles, together with a decrease in the lactacidogen but no change in the lactic acid of muscle. Seven units of insulin produced an increase of 60 per cent in the liver glycogen in guinea pigs which had received 3 grams of glucose just prior to the injection (50).

Cori (51) found that, in rabbits, within the first hour after the injection of insulin, there was no appreciable change in either the glycogen or the total carbohydrate content of the liver. In 2 to 6 hours it was found that the glycogen might remain constant or might decrease. In experiments on mice, in only 50 per cent of cases was the liver glycogen reduced and it was also found that insulin lowered the free liver sugar of starved rabbits and mice. It is assumed by this author that, in those cases in which the liver glycogen had been diminished by insulin, some special form of glycogenolysis occurred, in which sugar was not

an end product and that this form is in some way connected with the glycogen synthesis produced by insulin, whenever there is an excess of sugar in the body.

The continuous administration of insulin is reported by Allen, Bowie, Macleod and Robinson (52) to lead, in depancreatised dogs, after a preliminary disappearance of fat, to changes in the liver, in which fat is deposited in the cells of the periphery of the lobules, while at the centre the cells appear to be degenerated. In some cases death supervenes and amounts such as 39 per cent of fat are found in the liver. There is no evidence that a similar condition develops in diabetic human patients treated with insulin and it thus appears possible that the pancreas, in addition to its insulin producing function, also gives rise to some other internal secretion necessary for the physiological integrity of the liver.

g. The effect of insulin on the pancreatic islet tissue. Provided that the degeneration of the islet cells has not proceeded beyond certain limits, it appears that insulin treatment may induce some amount of recovery in the functional activity of the islet tissue. Copp and Barclay (53) report that, after two weeks of treatment with insulin, so regulated as to maintain complete freedom from hyperglycemia, regeneration of the β-cells in the islet tissue occurred in almost completely depancreatised animals. These findings are confirmed by Redish (54) and Visentini (55). It is emphasized that such results are only to be expected in cases where the disease is present in a mild or incipient form.

h. The effect of insulin on the nervous system. It is stated by Olmsted and Logam (56) that insulin acts on the bulbar centres and particularly the respiratory centre. It is also, according to Garrelon and Santenoise (34), a powerful excitant of the vagus. Gabbe (57) states that insulin convulsions in frogs do not occur after destruction of the spinal cord.

i. The relation between insulin and adrenalin. There appears to be no difference in the action of insulin in normal and in totally adrenalectomised animals. It is reported by Stewart and Rogoff (58) that neither intravenous nor subcutaneous injections affect the output of adrenalin in the cat and the authors conclude that there is no close relationship between the adrenals and the pancreas in carbohydrate metabolism. On the other hand, Sundberg (59) states that the action of insulin is more powerful after removal of the adrenals. The lethal dose for adrenalectomised rats is stated by Lewis (60) to be much smaller

than for intact animals, so that, according to the two latter authors, it would seem that there is some antagonism between insulin and the adrenal secretion. This is denied by Mueller, Lewi and Myers (61), who found that, on some occasions, adrenalin injected at the same time as insulin increased the action of the latter by lowering the blood sugar more than usual. In some cases, however, the action of insulin was slightly diminished for the first two hours and increased after four hours. It has already been mentioned that adrenalin and insulin are not antagonistic as regards phosphorus metabolism.

The formation of aldehydes by liver tissue has been studied by Gottschalk (62) who states that the action of insulin increases, while that of adrenalin diminishes it.

In a transfusion experiment carried out by Houssay, Lewis and Molinelli (63) it was found that the injection of insulin into the donor caused hyperglycemia in the recipient dog and this was attributed to the production of adrenalin and the action of this on the tissues of the transfused animal.

j. The effect of insulin on the respiratory exchange and metabolism. As a result of the injection of insulin, it was found by Dudley, Laidlaw, Trevan and Boock (64) that the intake of oxygen and the output of carbon dioxide were both lowered in mice, observations at temperatures of 18° and 30°C. giving similar results. The authors consider that the disappearance of sugar from the blood is not due to a direct increase of the rate of combustion nor to an increased glycogen storage in the liver. Dickson, Eadie, Macleod and Pember (65) found an increase in the respiratory quotient with an augmented oxygen consumption and respiratory volume but they were uncertain as to how far this rise is due to increased consumption of carbohydrates. That the effect of insulin is to increase both the consumption of oxygen and the excretion of carbon dioxide is agreed by Fuchs and Schill (66), Burn and Dale (67) and Laufberger (68). On the other hand, it is stated by Matton (69) that, in rabbits, the injection of insulin does not produce an increase in carbon dioxide at any stage of preconvulsive hypoglycemia. In doses insufficient to bring about hypoglycemia no noticeable variation in temperature or carbon dioxide output could be detected but Ambard, Schmidt and Arnovlyevitch (70) find that small doses increase the carbon dioxide output. In experiments upon isolated tissues, insulin appears to have no effect on the carbon dioxide output and, according to de Cloedt and v. Canneyt (71), it does not produce, in vivo, either an increase of carbohydrate metabolism or of heat pro-

duction. The latter opinions are confirmed by Heymans and Matton (72) and by Bouckaert and Stricker (73).

Kellaway and Hughes (74), however, in two experiments on normal individuals found a marked rise in the respiratory quotient after insulin which was due to increased carbon dioxide elimination, the oxygen intake remaining normal. This they considered to be due to the transformation of carbohydrate to some other complex with an accompanying economy in the use of oxygen and liberation of excess carbon dioxide. In a third experiment with a smaller dose of insulin a rise in the respiratory quotient was again observed but not to so great an extent. The rise in the respiratory quotient following insulin has also been observed and reported by Feyertag (75), Lyman, Nicholls and McCann (76), LaRoche and Tacquet (77) (two cases of hyperthyroidism and two cases of thyro-ovarian insufficiency), Gabbe, (78), Weiss and Reiss (79), DeBarenne and Burger (80) (in the decerebrate fasting cat), by Krogh and Brandt-Rehberg (81), who consider it to be mainly due to increased consumption of carbohydrate, thus differing from de Cloedt and his supporters mentioned above.

Poggio (82) reports that, in his experience, insulin has practically no influence on the respiratory exchange, but probably the most recent opinion on the subject is that of Lawn and Wolf (83) who state that, in the diabetic, the first action of insulin is to convert glucose into glycogen and then, after a reasonable supply of glycogen is provided, the insulin enables the body to mobilise and burn the glycogen in the normal way. After insulin, the authors state that the respiratory quotient falls on the ingestion of food and that this is not due to retention of carbon dioxide by the alkali liberated on the disappearance of ketones and acids from the blood.

There thus remains some amount of controversy on this question.

k. Insulin and heat production. In addition to what has already been reported in this connection, mention may be made of the work of Azuma and Hartree (84), who found that in isolated frog muscle insulin had no effect on the heat production, but that, in the intact frog, there was a pronounced rise after injection. Lyman, Nicholls and McCann (75) report a rise of 10 per cent in heat production, but Noyons, Bouckaert and Sierens (85) state that when the fall of temperature caused by insulin has reached 34°C. the heat production begins to diminish. It thus appears that insulin disturbs the power of heat regulation but the results are by no means constant.

l. Insulin and thyroid secretion. Thyroidectomised rabbits are more

susceptible to the action of insulin than normals. Ducheneau (86) reports that the fall in blood sugar is greater and the minimum lethal dose smaller in such animals and the general opinion is confirmed by Burn and Marks (87) who infer that the greatest cause of individual variation in the response of rabbits to insulin is a difference in the degree of thyroid activity and by Houssay and Busso (88), who state that thyroid feeding in rats restores their resistance to normal.

m. Certain miscellaneous reactions. There appears to be no evidence that the reduction of blood sugar by insulin is due to conversion into lactic acid. Servantie (89) states that insulin hypoglycemia is accompanied by a diminution instead of an increase in the lactic acid of the blood as suggested by Andova and Wagner (90).

After total pancreatectomy in dogs, the blood diastase falls, but subsequent injection of insulin causes it to rise slightly. It is suggested by Markowitz (91) that the hyperglycemia of diabetes is not due to excessive glycogenolysis but to a decreased diastatic activity of the blood.

Insulin reduces the excretion of ketones and ketonic acid (92). It has no action on the oxidation of aceto-acetic acid in phloridzinised animals and hypoglycemic convulsions are not due to ketosis from accumulated aceto-acetic acid (93). From an examination of fourteen patients, Killian (94) concludes that insulin temporarily inhibits the production of ketone bodies and thus readjusts the acid-base equilibrium of the blood.

Cori (95) considers that the reduction in blood sugar following insulin in phloridzin poisoning is due to storage and not oxidation.

Certain cardio-vascular changes have been noted by Edwards, Page and Brown (96) to follow the injection of insulin in some cases. Slight changes in the intra-arterial pulse curve, a lengthening of the period of developing tension in the ventricle and a decrease in the maximum pressure developed are mentioned and it is stated that, in some cases, intravenous injection of glucose improved the heart's action.

Insulin has no effect on the fermenting activity of B. coli (97), nor usually has it any effect on enzymes (98).

Finally, these miscellaneous reactions are concluded by the report, communicated by Bowie and Robinson (99), that, after the administration of insulin, near the site of injection, a marked degeneration of the muscle with leucocytic invasion has been noticed. It is also stated that other agencies, e.g., hypertonic glucose solution, have a similar effect.

XIII. THE EFFECT OF EXTERNAL FACTORS, DRUGS, ETC., ON THE SECRETION AND ACTION OF INSULIN. It is stated by Clark (1) that stimulation of the vagus causes a secretion of insulin and McCormick, Macleod and O'Brien (2) also report that there appears to be some indication of vagus control of insulin secretion. On the other hand, Banting and Gairns (3) consider that the formation of insulin is not under nervous control. Section of the vagus appears to diminish the sensitivity of an animal to insulin, while cutting the splanchnics has the opposite effect (4). These authors also find that removal of the suprarenals has a considerable augmenting influence on sensitivity to insulin and this is confirmed by Hallion and Gayet (5).

As regards the effects of drugs, etc., it is found that subcutaneous injection of pituitary extract diminishes or abolishes completely the hypoglycemia resulting from insulin administration (6). This is confirmed by Magenta and Biasotti (7) and by Housay and Magenta (8), who state that removal of the pituitary increases sensitivity to insulin. Other drugs which bring about the same effect are picrotoxin, strychnine, morphine and adrenalin (7). The same authors report that pilocarpine, caffeine, eserine, thyroid extract, nicotine and cocaine have no influence on the action of insulin. After ergotoxin, Burn (6) states that a small dose of insulin has a much greater effect than before and this fact, he considers, supports the view that, during insulin action, the liver attempts to replace from its store of glycogen the sugar which has disappeared from the blood.

Stewart and Rogoff (9) suggest that morphine hyperglycemia depends in some way on the integrity of the adrenals and find that it is counteracted by insulin.

The action of parathyroid extract given by the mouth, according to Forrest (10), produces a greater fall in blood sugar than that produced by insulin alone.

With further reference to the action of caffeine, reported above to be negative, Labbe and Thęodoresco (11) state that subcutaneous injection of caffeine and insulin together result in a modified hypoglycemia and these authors consider that the chemical action of caffeine on the blood is to some extent altered by insulin.

When insulin and alcohol are given by the mouth, it is stated by Blatherwick, Maxwell and Long (12) that a reduction in blood sugar takes place, which the authors attribute to the action of the alcohol, as insulin is not absorbed from the alimentary canal.

The actions of chloroform, ether and chloralose have been studied

in their relation to insulin by Mauriac and Aubertin (13). These authors found that, in the case of chloroform, experiments on three dogs showed that in one of these insulin was inactive, while in the other two, it produced the normal amount of hypoglycemia. In four rabbits anesthetised with chloroform, the action was also normal. In four healthy dogs, anesthetised with chloralose, insulin produced normal hypoglycemia in two cases, but in a third this condition was only transient and after an hour the blood sugar level was normal. In the fourth case, insulin was inactive. In three diabetic dogs, under the same anesthetic, the injection of insulin produced little or no effect. Of three rabbits, tested in the same way, two reacted normally, while the third showed only an insignificant amount of hypoglycemia.

Ether anesthesia previous to the administration of insulin rendered the latter inactive in three out of four dogs, the fourth being very little affected, but the action was normal in three rabbits.

The authors conclude that ether anesthesia exerts the most powerful counteraction to insulin and that, in making tests of insulin potency, it is advisable to dispense with the use of anesthetics as these tend to falsify the results.

The same authors also find that the action of atropine also renders insulin hypoglycemia quite inconstant in degree. It is suggested that insulin action cannot be explained on the basis of an increase in glycolysis, either in the tissues or in the body fluids and these authors consider that the pancreatic internal secretion has, as its principal rôle, the sensitising of the nervous system, permitting the metabolism of carbohydrates to be regulated thus.

Magenta and Biasotti (14) state that the action of insulin is antagonised by potassium chloride and that calcium chloride exerts a similar effect but to a less degree. Acetyl-cholin has no effect, while ergotoxin augments the action of insulin to some extent and disodium phosphate both augments and prolongs it. The antagonistic action of calcium chloride is confirmed by Sjollema and Seekles (15).

As might be expected, carbohydrate creates a higher requirement of insulin than either protein or fat and even if the same calorie equivalent of another foodstuff be administered, it is reported by Sherrill (16) to call for less insulin than the corresponding amount of carbohydrate.

Novoa-Santos (17) considers that secretin, in addition to its action on the pancreatic juice, also influences the pancreatic internal secretion, while Epstein and Rosenthal (18) hold the view that the controlling factor in the activity of insulin is trypsin. The latter authors

suggest that, in diabetes, the trypsin entering the blood may cause glycogenolysis to take place in the liver, at the same time inactivating the insulin produced in the pancreas.

From these notes it will be apparent that there is a variety of circumstances which may modify the action of insulin and it is emphasised that the individual variations in animals used for testing is a factor not without considerable influence.

XIV. THE PHYSIOLOGICAL ASSAY OF INSULIN. This is usually carried out with rabbits and it is essential, as Clough, Allen and Root (1) point out, that these should be normal in every sense of the word. Tests should not be carried out on animals with a low initial blood sugar content and insulin should not be given in doses producing a drop to below 0.07 per cent. The administration should be in proportion to the weight of the animal. If these precautions are carried out, the fall in blood sugar produced by a given product is absolute; it is independent of weight and initial sugar level and the authors claim that duplicate results can always be got within a variation of 0.014 gram. Penau and Simmonet (2) state that suitably prepared rabbits will give the same response to the injection of the same quantity of insulin and that differences of a quarter of a unit in the amount injected can be demonstrated.

Blatherwick, Long, Bell, Maxwell and Hill (3) find that rabbits are less resistant to insulin when on a diet of alfalfa hay than on a mixture of this and crushed barley. The authors explain this on the ground of the base forming action of alfalfa, the mixed diet being acid forming. They also state that to produce the same degree of hypoglycemia, the intravenous dose of insulin varies directly with the body weight and that convulsions are produced more quickly if the animal has previously experienced one or two. Putter (4) considers that the production of convulsions should be the principal guide in the standardisation of insulin.

As regards the use of white rats, it is reported by Voegtlin and Dunn (5) that the mortality of these animals is greatly increased if the room temperature reaches 28 to 30°C. It is needless to point out the importance of this in standardising.

White mice have also been used in this connection and it is stated by Cheadle (6) that 1 rabbit unit is equivalent to 167 mouse units, and by Bouckaert and Stricker (7) that the glucose equivalent of the clinical unit is 1.33 to 1.6 grams.

A comparison of the strength of several commercial insulin prepara-

tions has been made by Depisch, Hogler and Uberrack (8), who suggest that the Toronto unit should be defined as the smallest quantity of insulin which causes not only a fall of blood sugar to at least 0.045 per cent, but also convulsions in three-quarters of the animals used. These authors recommend that at least four experiments should be carried out on two or three animals.

Macleod and Orr (9) state that the potency of insulin is proportional to a/b, where a is the difference between the normal blood sugar and the average $1\frac{1}{2}$ to 3 to 5 hours after injection and b is the difference between the normal blood sugar and 0.045. The dose is made in proportion to the weight of the rabbit and no animals should be used whose blood sugar level is outside the limits 0.100 to 0.125 per cent.

It will probably be realised that the satisfactory biological assay of insulin presents some difficulties, but provided the precautions which have been outlined are taken and that sufficient animals are used to eliminate as far as possible the individual variation factor, there seems no reason to suppose that any grave fallacies are likely to arise with this method of standardisation.

XV. Some notes on insulin therapy. It is beyond the scope of this review to go deeply into the subject of treatment with insulin. It may, however, be interesting to record one or two points which have been brought out during the experience of the past year or two.

Several authors have published notes of their cases mainly in the professional journals and these speak for themselves. The main points which have emerged are, first, the need for caution, in order that hypoglycemic reactions may be avoided; secondly, that insulin treatment, to be effective, must not be applied in cases where there is a septic lesion. This must be cleared up before commencing the treatment and it will usually be found that the removal of the sepsis itself leads to an increase in carbohydrate tolerance. Of course, where coma is threatened, insulin should be given without delay (1).

The usual method of administration is subcutaneous injection, but in cases of coma insulin should be given intravenously. A considerable amount of work has been published dealing with the absorption of insulin from the alimentary canal, skin and other surfaces, but, for the most part, the records show that insulin applied in this way is ineffective. Thus, Thatcher (2) and Blatherwick, Maxwell and Long (3) report that insulin is not absorbed from the alimentary canal, whereas, on the other hand, Murlin, Sutter, Allen and Piper (4) state that insulin in crude solution containing 0.1 per cent HCl, when placed directly

in the duodenum of a diabetic, can be absorbed and cause utilisation of sugar. Insulin contained in "enteric coated" tablets designed to delay temporarily the action of trypsin has been found in some patients to survive the stomach, to be absorbed in the intestine and to improve the diabetic condition. Peskind, Rogoff and Stewart (5) find that insulin in serum, defibrinated blood or water is absorbed from the rectum of rabbits, with a rapid effect on the blood sugar. This, however, is less prolonged than after subcutaneous injection and relatively large doses are necessary in most cases. Insulin dissolved in saline has no effect when administered per rectum and no effect on dogs, normal or depancreatised, when given in water or serum.

The oral administration of insulin has been tested by Harrison (6), who states that double the quantity used for subcutaneous injection is ineffective in most cases. This is considered to be due to there being no way of controlling escape through the pyloric sphincter, or of estimating the amount destroyed by pepsin or absorbed. When an alcoholic solution of insulin is administered to rabbits by means of a stomach tube, Maxwell, Blatherwick and Sansum (7) find that this has no effect on the blood sugar level, but by a modification of the process of preparation these authors were able to obtain a product which, when administered in the same way to rabbits, caused a fall in blood sugar.

The oral administration of insulin mixed with an emulsion of olive oil was found by Salen (8) to be effective in two cases out of three examined and Levy and Cordier (9) also report inconstant results with this method of treatment. Blum (10) states that powdered insulin hydrochlorate is absorbed by the tongue and, if the preparation is readily soluble and very active, the same effect is produced as by subcutaneous injection of about one-third of the quantity. The results, however, are less constant.

Telfer (11) found that, in rabbits, crude insulin was effectively absorbed through the skin, while Stenstrom (12) states that attempts to administer insulin per rectum failed through inactivation by the feces. If, however, the latter were heated for a few minutes, it was found that inactivation did not take place. It was found by Fisher (13) that the absorption of insulin from the scrotal sac was rapid and the effect well maintained. From the vagina or the ileum the effect was prompt but temporary and the author states that the insulin is excreted by the kidneys. The same author, in collaboration with Noble (14), found that the insulin could be recovered from the urine by half saturation

with ammonium sulphate, the amount depending on the rate of absorption. After intravenous injection, it is stated that nearly all can be recovered, but less after vaginal or ileal administration and only one-sixth after subcutaneous injection.

It will thus be seen that there is very little practical value attached to methods of administration other than subcutaneous or intravenous injection, very inconstant results for the most part having been obtained. The most recent work on the subject has lately been completed by Muller and Corbitt (15) who find that the effect of insulin injection on the blood sugar content of normal rabbits is markedly increased if the intradermal method is used. Subcutaneous injections of the same amount into the same, as well as different, animals are less effective and a comparison of the intradermal, subcutaneous and intravenous methods, using equal amounts, shows that the effectiveness decreases in the order mentioned.

The degree and period of effectiveness does not apparently depend on the rapidity of resorption, but on a hitherto unknown factor, which the authors consider to be very probably related to the involuntary nervous system. This property seems to be inherent in the skin, rather than in the subcutaneous tissues or the body fluids.

With regard to the relief of hypoglycemic symptoms, Herring, Irvine and Macleod (16) point out that glucose and mannose are equally efficient. Maltose is less effective and slower in its action. Fructose and galactose are slightly active, but lactose, tetra-acetylglucose, tetramethylglucose, trimethylglucose, sucrose, α- and β-methylglucose, β-glucosan and glucose monoacetone are inactive. It is stated that salicyn may actually have a harmful effect and the authors suggest that a definite type of carbohydrate molecule may be required to antagonise insulin.

Further points relating to insulin therapy are referred to by Maclean (17), who states that induration of the body surface may be produced by continual injections in approximately the same spot. Thus, the site of injection should be varied as much as possible. The diets for patients under treatment with insulin usually contain a fairly large amount of fat. It is important that, if possible, carbohydrate should be given in moderate amount so as to ensure safety and render the diet more palatable.

If it is impracticable for blood sugar estimations to be carried out concurrently with insulin injections, the latter should be given so as to

keep the urine almost but not quite free from sugar, but if frequent blood sugar tests are available, it is desirable to administer an amount sufficient to reduce the sugar level to normal.

An important feature in patients undergoing insulin treatment is a tendency to put on fat. A direct formation of fat from sugar does not take place under the influence of insulin, but an intermediate product may be represented by the formation of a glucose-phosphoric acid combination (Embden's lactacidogen), and it is at all events certain that insulin has a profound effect on both fat and protein metabolism.

Patients under the influence of insulin should be kept under careful observation since its action is liable to be interfered with by otherwise trivial conditions, e.g., cold, a slight attack of influenza, mild gastric trouble, or even the administration of gas for removal of teeth. Furthermore, some patients appear to become accustomed to its injection, so that larger doses are necessary. This may indicate increased tolerance or a steady progression of the lesion.

Finally, as Maclean points out, insulin has not provided a substitute for the normal processes from a clinical aspect and at present its action must be regarded mainly as palliative: however, "its marvellous success in the treatment of severe and otherwise hopeless cases of diabetes is one of the most striking triumphs in the whole field of modern therapeutics."

XVI. Methods of estimating the blood sugar. Sixteen methods were outlined in the previous review and since 1923 a few more have been published. Amongst these may be mentioned Hagedorn and Jensen's (1), which is a micromethod employing only 0.1 cc. of blood and which is stated to give surprisingly concordant results. Haskins and Holbrook (2) have adapted Shaffer and Hartmann's copper iodine method to clinical use, while new methods have been described by Paton (3), Calvert (4) and Milroy (5). A modification of Bang's method has been proposed by Coppans (6) and Gilbert and Bode (7) have adapted Folin and Wu's method to the estimation of samples weighed on a torsion balance considering that pipettes are not reliable for small amounts of blood.

A comparison of methods has been carried out by Sweany and Johnson (8), who find that the picramic acid methods are unreliable in certain pathological conditions. It is said that phenol and potassium phenyl sulphate interfere and cause false results, but the authors state that Folin and Wu's method is satisfactory.

Lynch (9), in a comparison of methods including those of Bang,

Folin and Wu, Mackenzie Wallis and Gallagher, Calvert, Benedict, Hagedorn and Jensen and Maclean, prefer the latter, while Stepp (10), in a review of comparisons between reduction, polarisation, fermentation and osazone methods for blood sugar, strongly recommends the fermentation method for diabetic blood.

Thalhimer and Perry (11) have devised a method which embodies the estimation of the total sugar, the solution under investigation afterwards being inoculated with B. paratyphosus which decomposes glucose and not lactose. The latter is then estimated as such. The breakdown is stated to be most complete in dilute solutions (not above 0.3 per cent). The authors claim that the method is designed for the more complete determination of the substances which constitute the reducing material in normal and pathological body fluids. In this connection, it is well to bear in mind that the methods ordinarily used for the estimation of sugar in body fluids and particularly blood are based upon a compromise. It is generally assumed that the amount of sugar in the blood is proportional to the reducing power of a protein-free filtrate and for comparative purposes this may be satisfactory. It is as far as we can go with our present knowledge, but, from what has already been said in an earlier section of the review, it will be realised that much work remains to be done before it can definitely be established that the true state of affairs can be represented in such a way.

To return to individual methods, it is probably true to say that Maclean's enjoys a greater measure of popularity than most of the others described, except perhaps in America. It is as simple, if not simpler than any other; but, even so, it will generally be found that most practitioners prefer to send their patients or the blood samples to a laboratory for the estimations to be carried out. What is needed, is a method which the practitioner can make use of to satisfy himself as to whether the patient's blood is normal or not, without the bother of making up or obtaining complicated solutions and manipulating a colorimeter or carrying out titrations. Moreover, it is often impossible to send patients to a laboratory on account of the distance, and, for the same reason, the sending of blood samples is usually undesirable, if not entirely useless on account of the glycolysis which takes place during transit. It thus often happens that the practitioner is dependent for his knowledge of the patient's sugar metabolism on the extent of glycosuria, which conveys, as is now generally realised, a very unreliable impression, on account of individual variations in the renal threshold.

After work extending over a period of two years, and directed towards the production of a really simple and rapid method, the present author devised one depending upon the reduction of potassium ferricyanide to ferrocyanide, precipitation of the latter by ferric alum to ferric ferrocyanide and colorimetric estimation against a standard made up from pure glucose. While it was possible to obtain a reasonable degree of accuracy without the use of a colorimeter, it was recognised that this method had little to recommend it as compared with other existing methods and it was accordingly abandoned without publication.

Recently, the problem has been attacked again from another angle and a new method has been devised which, with a minimum of time, apparatus and manipulation, will enable the practitioner to determine whether hyperglycemia is present or not and, in addition, the degree of glycemia within certain limits. For practical purposes, it is relatively unimportant to be able to determine the amount of sugar present to the third decimal place, and, furthermore, it is extremely questionable whether any of the existing methods can legitimately claim to do this. What the medical man wants to know is whether the patient's blood is hyperglycemic, normal or, where insulin treatment is being carried out, hypoglycemic. The method referred to,[3] which is described in full elsewhere (12), tells him this and, in addition, provided the conditions are carefully controlled, which is a matter of no difficulty, the degree of glycemia between 0.075 and 0.3 per cent in intervals of 0.025.

It is felt that this method, which has been carefully tested, both with

[3] The proteins of the blood (0.2 cc.) are precipitated by heating in the presence of acid sodium sulphate and treatment with dialyzed iron (Maclean's method). Twenty cubic centimeters of the protein-free filtrate are transferred to a boiling tube, 2 drops of 20 per cent sodium hydroxide and 1 cc. of 0.0075 per cent methylene blue added and the mixture boiled. Immediately vigorous boiling commences, the time is noted and the boiling allowed to proceed as briskly as possible until the blue color disappears from the solution, when the time is again noted. Disappearance in 37 seconds indicates 0.3 per cent sugar; 60 seconds, 0.225 per cent; 1 minute, 25 seconds, 0.175 per cent; 1 minute, 55 seconds, 0.125 per cent; 2 minutes, 45 seconds, 0.075 per cent, etc., a graph being used to calculate the result.

If it is merely desired to know whether the blood has a normal concentration of sugar or not, the boiling should be allowed to continue for 2½ minutes; if at the end of this time the blue color has not disappeared, the sugar percentage is below 0.1.

Particular attention must be paid to accurate weighing and measurement of the methylene blue, and the boiling must be as vigorous as possible.

blood filtrates and standard glucose solutions, may be of considerable assistance to the country practitioner in diagnosis and in treatment. The apparatus and solutions required are reduced to a minimum and the time occupied in the whole process is not more than ten minutes.

SUMMARY

For the most part this review is not adapted for summarising, since each section really represents a summary in itself of the work published on the particular subject during the past two years. However, a brief indication of the scope of each section, together with any outstanding points, is now given.

1. The nature of the blood sugar, despite a considerable amount of fresh evidence, remains undetermined.

2. The distribution of sugar between corpuscles and plasma is still doubtful, but it seems, from the evidence available, that the greater part at least of the sugar is found in the plasma.

3. The concentration of sugar in arterial blood is usually slightly greater than that in venous blood and, ordinarily, no differences exist in this respect between venous and capillary blood.

4. The number and variety of physiological conditions which influence glycemia are considerable and individual variations also exert an effect in this connection.

5. The effect of different drugs, internal secretions and inorganic salts has been the subject of attention and the work published is detailed.

6. Variations in the blood sugar content have been noticed in diseases other than diabetes.

7. The factors which are responsible for the glycolysis which takes place when blood is allowed to stand are still obscure. Bacterial action is partly but not entirely concerned in this. The rate and degree of disappearance of the sugar are very inconstant and it is probable that a variety of factors take part in bringing about this condition. By bacterial action, oxidation to saccharic and mucic acids appears to take place, but if the action of bacteria is inhibited by toluol, the reducing power of the blood still disappears, although in this case the glycolysis seems to take a different path, since neither saccharic nor mucic acid appear to be formed under these circumstances. It is suggested by some observers that glycolysis is less in diabetic than in normal blood. This, however, seems to be denied by the majority of opinions and the author's experience in this connection points to there being no signifi-

cant differences between the two types of blood. Some work in connection with the effect of different anticoagulants is reported, but this is not at present sufficient for the formation of a definite opinion. The sugar content of protein-free filtrates does not appear to remain constant, in the author's experience, although it has been demonstrated to be so by another worker. A possible explanation for this discrepancy in results is offered.

8. Glucose tolerance tests are valuable in the diagnosis and treatment of diabetes and as these are simple to carry out and convey information which could be obtained in no other way, their employment should become a matter of routine practice.

9. Various points in connection with the preparation of insulin are detailed. Most of these are directed towards the attainment of a more efficient and cheaper product. The chemical properties, insofar as they have been worked out, are detailed. It has not, so far, been possible to determine the constitution of insulin.

10. Insulin may be obtained from a variety of sources, both animal and vegetable. The yield from fish is stated to be ten times as great as that from mammalian pancreas.

11. The work done upon the physiological effects of insulin is voluminous and ninety-nine authorities are quoted in this section alone, which is divided up by thirteen subtitles. These deal with the effects of insulin in relation to glycemia, blood-cells, blood-pressure and blood-volume, cerebrospinal fluid, phosphorus, lipoids, the liver, the pancreatic islet tissue, the nervous system, adrenalin, respiratory exchange and metabolism, heat production, thyroid secretion and with certain miscellaneous reactions.

12. The secretion of insulin and its action are modified by a variety of external factors amongst which may be mentioned drugs, including anesthetics, inorganic salts and the nervous system. The importance of allowing for individual variations in animals in interpreting results is emphasised.

13. Certain points dealing with the physiological assay of insulin are reviewed.

14. Mention is made of the more important features of insulin therapy, in the light of experience gained during the period of its practice.

15. Brief indications are given of recent developments in methods for blood sugar estimation. Where the apparatus and solutions are available several reliable methods exist, and of these, it is probable that Maclean's is the most popular. They are all, however, laboratory

methods and attention is drawn to one devised by the author, which is considerably simpler than any hitherto described and which it is hoped may be useful as a substitute where the carrying out of other methods is impracticable.

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